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# INVESTIGATION OF THE METABOLISM OF CHLORINATED HYDROCARBONS IN SUBHUMAN SPECIES

by

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The purpose of this research program was to provide metabolic data on four rather common drinking water contaminants. The compounds were 1,2,4-trichlorobenzene (TCB), bromodichloromethane (BDC), bis(2-chloroisopropyl) ether (BCIE) and bis(2-chloroethyl) ether (BCEE). The compounds, labeled with $^{14}C$, were administered orally and sometimes intravenously, to rats and rhesus monkeys. Studies on TCB included excretion and tissue distribution in rats, pharmacokinetic studies in monkeys and subchronic studies in monkeys. Daily oral doses of 174 mg/kg were uniformly fatal to monkeys in less than one month. Activity of liver enzymes was increased. Pharmacokinetics and tissue distribution of BDC was examined in rats and monkeys. The compound was excreted primarily via the lung either unchanged or as $CO_2$. BCIE in monkeys was also excreted via the lung and was very toxic to the eyes and kidneys following multiple 30 mg/kg oral doses. Preliminary studies of BCEE in two rhesus monkeys produced the same signs of periorbital toxicity as BCIE when oral doses of 10 mg/kg were administered.

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# FOREWORD

The many benefits of our modern, developing, industrial society are accompanied by certain hazards. Careful assessment of the relative risk of existing and new man-made environmental hazards is necessary for the establishment of sound regulatory policy. These regulations serve to enhance the quality of our environment in order to promote the public health and welfare and the productive capacity of our Nation's population.

The complexities of environmental problems originate in the deep interdependent relationships between the various physical and biological segments of man's natural and social world. Solutions to these environmental problems require an integrated program of research and development using input from a number of disciplines. The Health Effects Research Laboratory, Research Triangle Park, NC and Cincinnati, OH, conducts a coordinated environmental health research program in toxicology, epidemiology, and clinical studies using human volunteer subjects. Wide ranges of pollutants known or suspected to cause health problems are studied. The research focuses on air pollutants, water pollutants, toxic substances, hazardous wastes, pesticides, and non-ionizing radiation. The laboratory participates in the development and revision of air and water quality criteria and health assessment documents on pollutants for which regulatory actions are being considered. Direct support to the regulatory function of the Agency is provided in the form of expert testimony and preparation of affidavits as well as expert advice to the Administrator to assure the adequacy of environmental regulatory decisions involving the protection of the health and welfare of all U.S. inhabitants.

This report provides metabolic data, in rats and rhesus monkeys, on four drinking water contaminants: 1,2,4-trichlorobenzene, bromodichloromethane, bis(2-chloroisopropyl) ether and bis(2-chloroethyl) ether. Pharmacokinetic and metabolic data on the adsorption, distribution and elimination of the parent compound and its metabolites following oral and intravenous administration allows one to predict whether the chemical or its metabolites accumulate in the body. Comparative pharmacokinetic studies in subhuman species are needed in the extrapolation of toxicological data from animals to man.

F. Gordon Hueter, Ph.D.
Director
Health Effects Research Laboratory

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# ABSTRACT

The purpose of this research program was to provide metabolic data on four rather common drinking water contaminants. The compounds were 1,2,4-trichlorobenzene (TCB), bromodichloromethane (BDC), bis(2-chloroisopropyl) ether (BCIE) and bis(2-chloroethyl) ether (BCEE). The compounds, labeled with $^{14}C$, were administered orally and sometimes intravenously, to rats and rhesus monkeys.

Studies on TCB included excretion and tissue distribution in rats following oral and intravenous doses. Pharmacokinetic parameters of TCB were examined in rhesus monkeys. Subchronic studies on orally administered TCB were conducted in monkeys. Daily oral doses of 25 mg/kg or less had no observable effects on monkeys. Liver biopsies indicated further that such doses had little effect on the drug metabolizing or mixed function oxidase (MFO) activity of the liver. Using doses of 90 and 125 mg/kg one of four monkeys on each of the dosage levels died but whether TCB toxicity was the cause of death was uncertain. Daily doses of 174 mg/kg killed two monkeys and would have been uniformly fatal if continued beyond one month. Recovery from toxic effects was rapid and appeared to be complete. TCB in doses of 90 to 174 mg/kg produced significant increases in the levels of four MFO enzymes in the primate liver studied _in vitro_. The effect of TCB on the _in vivo_ metabolism of chlorguanide was also investigated. The metabolic fate of this compound can be used to estimate the level and type of hepatic MFO activity. The results of this _in vivo_ test of MFO activities correlated well with the _in vitro_ measurements of hepatic enzyme levels.

The pharmacokinetics and tissue distribution of BDC was examined in rats and monkeys. BDC was excreted primarily via the lung as parent compound or $CO_2$. The maximum amount recovered in the urine was 1% in rats and 6% in monkeys. Monkeys excreted 0.5 to 1.5% in feces.

Pharmacokinetic studies of BCIE following oral and intravenous administration in rhesus monkeys showed that only about one-third of the administered radio-activity was recovered in the urine and feces, therefore, one of the primary routes of excretion must have been via the lungs. BCIE was very toxic to the eyes and kidneys when multiple 30 mg/kg doses were administered. Preliminary studies of BCEE in two rhesus monkeys produced the same signs of periorbital toxicity observed with BCIE; in monkeys BCEE was more toxic than BCIE.

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## SECTION 1

## INTRODUCTION

In the volume "Drinking Water and Health" the first summarized recommendation for additional research lists "Studies of the physiological and biochemical mechanisms by which the toxic substances in water produce their effects" (1). We have approached this objective by studying the metabolic fate and pharmacokinetics of four of the common contaminants of chlorinated drinking water. The compounds chosen include trichlorobenzene, a bromine analogue of chloroform and two bis-2-chloroalkyl ethers. We have used rats and monkeys with special emphasis on the use of subhuman primates since studies by the author and by many other investigators support the hypothesis that in general the metabolic fate of compounds in monkeys more closely simulates the fate in man than do corresponding data obtained in other common laboratory animals such as dogs or rodents (C. C. Smith, in press).

Although there are some data on the metabolic fate of 1,2,4-trichlorobenzene in rats and the effects of this compound on metabolism of foreign compounds (2,3) the data obtained in this study were designed to corroborate and extend these previous studies.

In the case of the chloroform analogue and the two bischloroalkyl ethers, very little previous data were available. Very recently Lingg *et al.*, have published data on the metabolic fate of bis(2-chloroethyl)ether and bis(2-chloroisopropyl)ether after single oral doses in rats (4).

When these studies were undertaken, it was hoped that the data they would provide using subhuman primates would be useful in judging the potential toxicity of these materials in man.

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# SECTION 2

## CONCLUSIONS

These studies on the metabolism of four halogen-containing water contaminants have shown certain characteristics and differences in metabolic patterns and associated toxic effects. Trichlorobenzene (TCB) is probably the least toxic compound when administered chronically but is probably the only compound with the potential of inducing liver drug-metabolizing enzymes. The compound has toxic effects at daily doses in excess of 125 mg/kg and is lethal in about 25 days when the daily oral dose is 174 mg/kg. The data on effect of TCB on chlorguanide metabolism demonstrated in this study suggest that measurement of the ratio of chlorguanide metabolites after single oral doses can be used as a method for estimating the ratio of P-450/P-448 enzyme activities *in vivo*.

Bromodichloromethane appears to be rapidly metabolized or exhaled but the amount of labeled materials excreted in the urine appears to be greater than occurs following chloroform administration. This is almost certainly the result of the greater lability of the Br-C bond compared to the Cl-C bond.

The two bischloroethers appear to be exhaled either unchanged or as $CO_2$. The occurrence of periocular irritation following single oral doses suggests that significant amounts of the parent compounds or active metabolites are being exhaled. Bis(2-chloroethyl)ether is probably slightly more toxic than its isopropyl analogue, bis(2-chloroisopropyl)ether.

## SECTION 3

## RECOMMENDATIONS

The results on trichlorobenzene (TCB) obtained in this study correlating the _in vivo_ measurement of MFO activities and P-450/P-448 ratios using chlorguanide with _in vitro_ measurements of typical MFO enzyme activities suggest that this procedure merits further trial as a possible method for estimating the state of hepatic drug metabolizing enzymes _in vivo_ in human subjects. Although an attempt was made to test the effect of a typical P-448 inducer (3-methylcholanthrene) the experiment provided inconclusive data and should be repeated. The data on hepatic enzyme induction in monkeys produced by TCB suggests that an attempt be made to see if TCB induces the same pattern of response in humans.

The data on bromodichloromethane (BDC) and on the two chloroethers are interesting and suggest the need for additional studies. Specific goals of these studies should be to measure the chronic toxicity of BDC in rodents and subhuman primates and to define the nature of the urinary metabolites.

More extensive metabolic and chronic toxicity studies of the two chloroalkylethers studied in this report are necessary to decide whether the renal and periorbital toxicity of these compounds is due to the parent compound or to a more active metabolite. There is a suggestion in these limited studies that these chloroethers may affect the menstrual cycle in monkeys and this possible side effect requires better definition. Since these chloroalkyl ethers are unstable, future studies should include special precautions to insure that the results obtained are due to defined compounds and not to the effects of undefined decomposition or degradation products.

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# SECTION 4

## IN VIVO STUDIES ON TRICHLOROBENZENE

### I. EXCRETION AND TISSUE DISTRIBUTION OF 1,2,4-TRICHLOROBENZENE (TCB) FOLLOWING SINGLE ORAL DOSES IN RATS

The purposes of this study were to determine the excretion and tissue distribution of TCB and metabolites in rats following single oral doses and to compare these results with those obtained when the compound was administered intravenously.

### Materials and Methods

Seven groups of four young (300-350 g) Charles River male albino rats were housed in stainless steel metabolism cages permitting separation of urine and feces. One group of four was killed at 3,6,12,24,48,72, and 96 hours post-treatment.

Treatment solution contained 2.0 mg (0.45 µCi $^{14}$C) 1,2,4-TCB per ml of water:ethanol:Emulphor®* (8:1:1,v/v/v). Each rat was given this solution by oral gavage in a dose of 10 mg/kg.

Urine and feces were collected at 24 hour intervals or at necropsy. Aliquots of urine were prepared immediately after collection for liquid scintillation counting (LSC) and the remainder of each sample stored at -20°C. Feces were also stored at -20°C until analyzed. The following tissues were analyzed by LSC for total $^{14}$C content: whole blood, plasma, liver, spleen, kidneys, lungs, heart, testes, brain, fat, muscle, and skin. In addition, the gastrointestinal tract was divided into four sections: stomach, small intestine, cecum, and large intestine; each section together with its contents was analyzed for total $^{14}$C activity.

### Results

The results of these studies expressed as percent of $^{14}$C dose administered are summarized in Tables 1 to 4. From Table 1 it is obvious that 80 to 90% of the $^{14}$C from TCB was excreted in the urine while 10 to 20% was recovered in the feces. Excretion of $^{14}$C was almost complete by 24 hours

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*Emulphor-EL-620® was obtained from the GAF Corporation and consists of a polyoxyethylated vegetable oil soluble in water.

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and was complete by 48 hours. One can estimate the $T_{1/2}$ of TCB in rats following oral doses to range from four to six hours. This fast or $\alpha$ phase is followed by a much longer slow $\beta$ phase.

Data on tissue distribution of TCB and metabolites (as $^{14}C$) are presented in Table 2. The highest tissue concentrations were observed at three hours. By 24 hours most tissues except the GI tract contained less than 3 µg/g as $^{14}C$. In percent dose, the tissues, other than the GI tract, which accounted for most of the TCB-derived $^{14}C$ were blood, liver, and kidney (Table 3). At 24 hours there was very little $^{14}C$ in the stomach, about 3.5% in the rest of the GI tract, and only 1% in all other tissues. The small amounts in the stomach compared to the persistence of $^{14}C$ in the rest of the GI tract suggested that the compound and its metabolites were not actively secreted by the stomach, but may have been actively secreted via the bile. Some radioactivity persisted in the fat, kidney, and liver for at least 96 hours after single oral doses.

Data on excretion, tissue distribution, and total recovery are summarized in Table 4. Essentially all the administered $^{14}C$ was accounted for in the urine, feces, and analyzed tissues after 12 hours. The $^{14}C$ not accounted for during the early periods after treatment was probably present in the fat and other pools, presently unidentified, which provided temporary depots until the levels in blood, liver, lung, etc., began to fall due to metabolism and urinary excretion of metabolites. Probably less than 2% of the $^{14}C$ administered persists in the rat beyond 48 hours.

## II. EXCRETION AND TISSUE DISTRIBUTION OF 1,2,4-TRICHLOROBENZENE (TCB) FOLLOWING SINGLE INTRAVENOUS DOSES IN RATS

The purposes of this study were to (1) determine the excretion and tissue distribution of TCB in rats following single intravenous doses and (2) compare these data to the results obtained following single oral doses.

### Materials and Methods

Seven groups of four young (300-350 g) Charles River male albino rats were used. The rats were housed in stainless steel metabolism cages permitting separation of urine and feces. One group of four rats was killed at 3,6,12,24,48,72 and 96 hours post-treatment.

The treatment solution was prepared by adding 193 µl (280.7 mg) of unlabeled 1,2,4-TCB and 150 µl (218.1 mg, 84 µCi) of $^{14}C$-labeled 1,2,4-TCB to 10 ml of a 1:1 (v/v) mixture of Emulphor® and absolute alcohol; this solution was diluted to 50 ml with distilled $H_2O$. The final treatment solution contained 10 mg 1,2,4-TCB (1.68 µCi $^{14}C$) per ml.

One femoral vein of each rat was exposed under light ether anesthesia and injected with a dose of 10 mg TCB (labeled as above) per kg body weight.

Urine, feces, and tissues were handled and assayed for $^{14}C$ acti[illegible] using the same procedures described previously.

## Results

The results of these studies expressed as µg TCB/gram tissue or percent dose (as $^{14}C$) are summarized in Tables 5 to 8. As shown in Table 5, excretion of $^{14}C$ was primarily via the urine (83-86%) and about 12% appeared in the feces. Excretion of $^{14}C$ was 90% complete by 24 hours and virtually 100% by 48 hours.

Data on tissue distribution of TCB and metabolites are presented in Tables 6 and 7. The data in Table 6 indicate that maximum tissue levels of $^{14}C$ occurred in some tissues at six hours (blood and plasma, liver and kidney) while in the other tissues the peak occurred at three hours or the three and six hour values were equal. Concentrations were higher in fat than in any other tissue analyzed. After 24 hours concentrations were higher than 1 µg/g in fat, kidney, and liver. Levels of $^{14}C$ were most persistent in the fat but at 96 hours were still appreciable in kidney and liver.

The data in Table 7 point out the importance of the blood compartment, liver, and kidney in the pharmacokinetics of TCB in the rat. Since the drug was administered intravenous, the prominent radioactivity in the small intestine, cecum and large intestine demonstrate the biliary excretion of TCB and/or metabolites and the probability of enterohepatic circulation (see GI tract data in Table 8).

In summary, after intravenous doses of $^{14}C$-labeled TCB, 85 to 90% of the $^{14}C$ appears in the urine with only about 10 to 15% appearing in the feces. There was early accumulation of radioactivity (assumed to be parent compound) in the fat and $^{14}C$ activity persisted in this tissue longer than in any other. Only about 2% of the dose was present in the body at 24 hours and much less at later periods.

## Discussion

If we compare these data after intravenous injection with the previous data following equivalent oral doses, we note that the overall metabolic patterns are quite similar following the two routes. The intravenous data demonstrate 1) that the compound undergoes active enterohepatic circulation, and 2) that the tissue levels are higher after intravenous injection. This is particularly evident when we compare the tissue levels (as $^{14}C$ in µg/g) at six hours in the fat, liver, spleen, kidney, lung, heart, brain, muscle, and skin. Nevertheless, the overall excretion patterns in urine and feces are essentially the same.

TABLE 1. CUMULATIVE EXCRETION OF 1,2,4 - TRICHLOROBENZENE (AS $^{14}C$) IN MALE RATS

SINGLE ORAL DOSE OF 10 MG/KG

| Collection Period Hours | Cumulative Excretion in Per Cent Dose Urine | Feces | Urine and Feces |
|---|---|---|---|
| 0-3 | 12.5 ± 5.4 (3) | 0.4 (1) | 12.9 |
| 0-6 | 37.8 ± 20.0 (4) | 0.2 (2) | 38.0 |
| 0-12 | 79.7 ± 7.8 (4) | 2.1 ± 1.1 (4) | 81.8 |
| 0-24 | 84.5 ± 6.8 (16) | 11.2 ± 0.8 (4) | 95.7 |
| 0-48 | 87.2 ± 5.4 (12) | 14.3 ± 0.4 (4) | 101.5 |
| 0-72 | 87.3 ± 5.0 (8) | 14.7 ± 0.6 | 102.0 |
| 0-96 | 85.1 ± 5.4 (4) | 14.9 ± 0.7 | 100.0 |

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TABLE 2. TISSUE DISTRIBUTION OF 1,2,4 - TRICHLOROBENZENE (AS $^{14}C$) IN MALE RATS

SINGLE ORAL DOSE OF 10 MG/KG

| Tissue | µg/g at Various Hours Post-Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3 | 6 | 12 | 24 | 48 | 72 | 96 |
| **Blood** | 5.7[a]<br>1.0-11.3 | 2.7<br>2.5-4.0 | 1.1<br>0.65-1.5 | 0.42<br>0.20-0.68 | 0.09<br>0.02-0.20 | 0.03<br><0.01-0.04 | <0.02<br><0.01-0.05 |
| **Plasma** | 10.4<br>1.8-20.9 | 5.1<br>3.9-6.8 | 1.7<br>0.94-2.4 | 0.62<br>0.32-0.92 | 0.15<br>0.06-0.24 | 0.05<br>0.03-0.07 | 0.03<br>0.02-0.06 |
| **Liver** | 8.1<br>1.9-15.7 | 4.6<br>4.1-5.2 | 2.1<br>1.5-2.9 | 1.1<br>0.76-1.5 | 0.59<br>0.45-0.79 | 0.38<br>0.27-0.44 | 0.32<br>0.22-0.44 |
| **Spleen** | 0.92<br>0.11-2.0 | 0.55<br>0.27-0.69 | 0.29<br>0.14-0.51 | 0.19<br>0.11-0.28 | 0.11<br>0.06-0.19 | 0.03<br><0.01-0.07 | 0.02<br><0.01-0.04 |
| **Kidney** | 31.7<br>7.1-64.8 | 22.4<br>17.0-25.4 | 6.2<br>4.1-7.4 | 2.4<br>1.7-3.4 | 0.86<br>0.43-1.3 | 0.57<br>0.37-0.88 | 0.29<br>0.18-0.37 |
| **Lung** | 3.0<br>0.57-6.2 | 1.6<br>1.3-2.0 | 0.71<br>0.48-0.96 | 0.45<br>0.27-0.61 | 0.21<br>0.15-0.33 | 0.08<br>0.03-0.12 | 0.04<br><0.01-0.08 |
| **Heart** | 2.1<br>0.45-3.4 | 1.1<br>0.65-1.6 | 0.59<br>0.40-0.96 | 0.33<br>0.12-0.52 | 0.18<br>0.09-0.25 | 0.05<br>0.02-0.13 | 0.03<br><0.01-0.07 |
| **Testes** | 1.1<br>0.19-2.3 | 0.67<br>0.50-0.82 | 0.33<br>0.24-0.49 | 0.18<br>0.06-0.33 | 0.08<br>0.03-0.17 | 0.07<br><0.01-0.11 | 0.03<br><0.01-0.05 |
| **Brain** | 0.52<br>0.13-0.76 | 0.27<br>0.23-0.29 | 0.15<br>0.10-0.24 | 0.12<br>0.06-0.17 | 0.06<br><0.01-0.12 | 0.05<br><0.01-0.07 | 0.03<br><0.01-0.06 |
| **Fat** | 17.2<br>4.6-24.4 | 13.8<br>6.8-17.1 | 4.8<br>2.7-6.8 | 2.8<br>2.0-3.8 | 1.5<br>0.87-2.6 | 0.80<br>0.57-0.99 | 0.62<br>0.33-0.90 |

(continued)

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TABLE 2 (continued)

| Tissue | μg/g at Various Hours Post-Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3 | 6 | 12 | 24 | 48 | 72 | 96 |
| Muscle | 0.74<br>0.09-1.5 | 0.49<br>0.32-0.83 | 0.17<br>0.06-0.31 | 0.09<br>0.01-0.14 | 0.05<br>0.02-0.09 | 0.05<br><0.01-0.07 | 0.03<br><0.01-0.05 |
| Skin | 2.78<br>0.64-4.6 | 1.24[b]<br>1.06-1.34 | 0.83<br>0.34-1.4 | 0.43<br>0.22-0.83 | 0.31<br>0.15-0.44 | 0.26<br>0.15-0.34 | 0.15<br>0.09-0.26 |

[a] Average of 4 animals and range.

[b] Average of 3 animals and range.

## TABLE 3. TISSUE DISTRIBUTION OF 1,2,4 - TRICHLOROBENZENE (AS $^{14}C$) IN PER CENT DOSE

SINGLE ORAL DOSE OF 10 MG/KG

MALE RATS

| Tissue | Per Cent Dose At Various Hours Post-Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3 | 6 | 12 | 24 | 48 | 72 | 96 |
| Blood | 4.8<br>0.82-9.3 | 2.6<br>2.2-3.2 | 0.94<br>0.59-1.3 | 0.36<br>0.15-0.57 | 0.09<br>0.02-0.19 | 0.02<br><0.01-0.04 | 0.02<br><0.01-0.05 |
| Liver | 2.8<br>0.79-4.8 | 1.5<br>1.4-1.6 | 0.76<br>0.60-0.95 | 0.39<br>0.28-0.49 | 0.25<br>0.16-0.37 | 0.15<br>0.11-0.19 | 0.12<br>0.09-0.16 |
| Kidney | 2.3<br>0.56-4.2 | 1.9<br>1.4-2.4 | 0.48<br>0.32-0.62 | 0.19<br>0.11-0.26 | 0.07<br>0.03-0.11 | 0.05<br>0.03-0.07 | 0.02<br>0.01-0.03 |
| Lung | 0.13<br>0.02-0.28 | 0.07<br>0.06-0.08 | 0.03<br>0.02-0.04 | 0.02<br>0.01-0.03 | 0.01<br><0.01-0.02 | <0.01 | <0.01 |
| Stomach | 6.2<br>0.3-9.9 | 0.50<br>0.05-1.4 | 0.07<br>0.05-0.09 | 0.05<br>0.02-0.08 | 0.02<br>0.01-0.03 | 0.01<br>0.01-0.02 | 0.01<br>0.01-0.01 |
| Small Intestine | 13.4<br>5.2-21.5 | 8.0<br>6.0-9.3 | 4.1<br>2.2-5.8 | 1.1<br>0.46-1.6 | 0.17<br>0.12-0.24 | 0.07<br>0.04-0.08 | 0.06<br>0.04-0.09 |
| Cecum | 1.2<br>0.06-2.4 | 3.2<br>1.5-5.6 | 3.1<br>2.4-3.7 | 1.1<br>0.5-2.7 | 0.20<br>0.14-0.29 | 0.05<br>0.04-0.06 | 0.05<br>0.03-0.08 |
| Large Intestine | 0.23<br>0.06-0.33 | 1.6<br>0.23-2.9 | 4.7<br>2.6-6.3 | 1.3<br>0.5-3.9 | 0.23<br>0.05-0.47 | 0.05<br>0.03-0.10 | 0.07<br>0.02-0.17 |
| Other Tissues | 0.23<br>0.06-0.44 | 0.12<br>0.09-0.16 | 0.07<br>0.04-0.10 | 0.04<br>0.01-0.06 | 0.02<br>0.01-0.04 | 0.01<br><0.01-0.01 | <0.01 |

### TABLE 4. RECOVERY OF 1,2,4 - TRICHLOROBENZENE (AS $^{14}C$) IN MALE RATS

SINGLE ORAL DOSE OF 10 MG/KG

| | Per Cent Dose at Various Hours Post-Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3* | 6 | 12 | 24 | 48 | 72 | 96 |
| Urine | 12.5 | 37.8 | 79.7 | 86.4 | 88.4 | 89.9 | 85.0 |
| Feces | 0.4 | 0.2 | 2.1 | 11.2 | 14.3 | 14.7 | 14.9 |
| G-I Tract | 21.0 | 13.3 | 12.1 | 3.6 | 0.6 | 0.2 | 0.2 |
| Tissues | 10.2 | 6.1 | 2.3 | 1.0 | 0.4 | 0.3 | 0.2 |
| Total Recovery | 44.1 | 57.4 | 96.2 | 102.2 | 103.7 | 105.1 | 100.3 |

* 4 rats per time period.

TABLE 5. CUMULATIVE EXCRETION OF 1,2,4 - TRICHLOROBENZENE (AS $^{14}C$) IN MALE RATS

SINGLE INTRAVENOUS DOSE OF 10 MG/KG

| Collection Period Hours | Cumulative Excretion in Per Cent of Dose Mean and Standard Deviation Urine | Feces | Urine and Feces |
|---|---|---|---|
| 0-3 | 6.0 ± 1.5 (4)[a] | <0.1 (1) | 6.0 ± 1.5 (4) |
| 0-6 | 31.1 ± 4.2 (4) | 0.1 (2) | 31.1 ± 4.2 (4) |
| 0-12 | 56.0 ± 11.2 (4) | 0.1 (4) | 57.6 ± 9.1 (4) |
| 0-24 | 77.6 ± 6.6 (16) | 7.0 ± 3.6 (16) | 84.6 ± 5.8 (16) |
| 0-48 | 83.2 ± 6.6 (12) | 11.5 ± 3.2 (12) | 94.7 ± 4.4 (12) |
| 0-72 | 86.0 ± 5.0 (8) | 11.0 ± 2.6 (8) | 97.1 ± 3.7 (8) |
| 0-96 | 83.2 ± 4.7 (4)[b] | 12.4 ± 1.9 (4) | 95.5 ± 3.3 (4) |

[a] Number of animals.

[b] One animal with low urinary excretion--may have lost some of the 0-24 hour sample.

TABLE 6. TISSUE DISTRIBUTION OF 1,2,4 - TRICHLOROBENZENE (AS $^{14}C$) IN MALE RATS

SINGLE INTRAVENOUS DOSE OF 10 MG/KG

| Tissue | µg/g at Various Hours Post Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3 | 6 | 12 | 24 | 48 | 72 | 96 |
| Blood | 3.70<br>3.18-3.94 | 4.88<br>4.40-5.74 | 2.66<br>1.91-3.32 | 0.74<br>0.54-0.91 | 0.23<br>0.17-0.34 | 0.09<br>0.09-0.20 | 0.09<br>0.04-0.17 |
| Plasma | 3.79<br>3.43-4.55 | 7.02<br>6.17-8.08 | 3.47<br>2.31-4.34 | 0.87<br>0.57-1.20 | 0.17<br>0.07-0.30 | 0.09<br>0.03-0.14 | 0.06<br>0.04-0.09 |
| Liver | 5.47<br>5.16-5.93 | 5.74<br>5.10-6.03 | 3.58<br>2.58-4.55 | 1.43<br>0.90-1.97 | 0.69<br>0.44-0.99 | 0.52<br>0.38-0.70 | 0.45<br>0.34-0.52 |
| Spleen | 0.82<br>0.69-0.95 | 0.82<br>0.72-0.89 | 0.92<br>0.61-1.50 | 0.35<br>0.28-0.44 | 0.47<br>0.32-0.79 | 0.16<br>0.13-0.19 | 0.13<br>0.08-0.20 |
| Kidney | 16.6<br>15.2-17.5 | 32.2<br>22.8-36.7 | 14.5<br>10.0-20.9 | 2.99<br>2.38-3.54 | 1.29<br>0.57-1.84 | 0.56<br>0.33-0.70 | 0.57<br>0.35-0.95 |
| Lung | 2.56<br>2.06-3.65 | 2.17<br>1.95-2.32 | 1.40<br>1.12-2.12 | 0.52<br>0.41-0.60 | 0.46<br>0.37-0.54 | 0.21<br>0.17-0.24 | 0.25<br>0.17-0.50 |
| Heart | 2.04<br>1.68-2.27 | 1.78<br>1.60-1.96 | 1.27<br>0.91-1.78 | 0.50<br>0.37-0.60 | 0.52<br>0.42-0.66 | 0.19<br>0.13-0.24 | 0.20<br>0.11-0.35 |
| Testes | 0.95<br>0.85-1.01 | 0.89<br>0.79-1.00 | 0.85<br>0.59-1.16 | 0.31<br>0.26-0.38 | 0.29<br>0.26-0.33 | 0.10<br>0.05-0.17 | 0.13<br>0.08-0.19 |
| Brain | 1.40<br>1.13-1.67 | 0.62<br>0.53-0.70 | 0.56<br>0.43-0.71 | 0.26<br>0.17-0.34 | 0.33<br>0.19-0.50 | 0.15<br>0.07-0.22 | 0.10<br>0.05-0.16 |
| Fat | 51.6<br>36.7-64.7 | 41.9<br>29.6-47.1 | 22.8<br>19.3-24.3 | 5.98<br>2.92-10.9 | 1.56<br>0.80-2.26 | 0.98<br>0.71-1.22 | 0.80<br>0.40-1.49 |
| Muscle | 1.63<br>0.96-2.93 | 1.20<br>0.97-1.40 | 0.78<br>0.42-1.04 | 0.32<br>0.23-0.58 | 0.33<br>0.29-0.40 | 0.11<br>0.04-0.15 | 0.11<br>0.09-0.12 |
| Skin | 3.24<br>1.36-4.62 | 1.65<br>1.36-1.91 | 1.11<br>0.92-1.31 | 0.45<br>0.34-0.51 | 0.58<br>0.45-0.82 | 0.18<br>0.07-0.34 | 0.16<br>0.12-0.20 |

TABLE 7. TISSUE DISTRIBUTION OF 1,2,4 - TRICHLOROBENZENE (AS $^{14}C$) IN PER CENT DOSE

SINGLE INTRAVENOUS DOSE OF 10 MG/KG

MALE RATS

| Tissue | Per Cent Dose at Various Hours Post-Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3 | 6 | 12 | 24 | 48 | 72 | 96 |
| Blood | 3.38<br>3.07-3.61 | 4.43<br>4.12-4.93 | 2.16<br>1.59-2.65 | 0.64<br>0.47-0.79 | 0.21<br>0.15-0.31 | 0.13<br>0.09-0.19 | 0.09<br>0.04-0.16 |
| Liver | 2.30<br>2.10-2.72 | 2.20<br>2.01-2.40 | 1.11<br>0.95-1.39 | 0.49<br>0.39-0.48 | 0.27<br>0.19-0.33 | 0.22<br>0.18-0.27 | 0.20<br>0.18-0.22 |
| Kidney | 1.17<br>1.07-1.26 | 2.23<br>1.77-2.86 | 1.03<br>0.75-1.64 | 0.23<br>0.19-0.28 | 0.10<br>0.04-0.13 | 0.04<br>0.03-0.06 | 0.04<br>0.03-0.08 |
| Lung | 0.10<br>0.09-0.13 | 0.09<br>0.08-0.10 | 0.06<br>0.04-0.09 | 0.02<br>0.02-0.03 | 0.02<br>0.02-0.03 | 0.01<br><0.01-0.01 | <0.01<br>(<0.01) |
| Stomach | 0.57<br>0.40-1.00 | 0.34<br>0.17-0.49 | 0.34<br>0.17-0.48 | 0.09<br>0.05-0.13 | 0.03<br><0.01-0.07 | 0.02<br><0.01-0.04 | 0.02<br>0.01-0.05 |
| Small Intestine | 9.41<br>8.78-10.1 | 15.8<br>13.4-18.5 | 4.31<br>3.56-5.24 | 1.26<br>1.01-1.56 | 0.21<br>0.05-0.37 | 0.09<br>0.03-0.13 | 0.07<br>0.05-0.10 |
| Cecum | 0.35<br>0.25-0.60 | 3.55<br>2.27-4.42 | 4.83<br>1.97-6.90 | 1.80<br>0.92-2.12 | 0.25<br>0.18-0.35 | 0.06<br>0.02-0.11 | 0.03<br>0.02-0.05 |
| Large Intestine | 0.34<br>0.19-0.53 | 0.79<br>0.56-1.22 | 4.38<br>2.90-6.34 | 2.06<br>0.60-3.16 | 0.29<br>0.07-0.79 | 0.15<br>0.04-0.52 | 0.03<br>0.02-0.05 |
| Other Tissues | 1.93<br>1.53-2.26 | 1.59<br>1.16-1.68 | 0.81<br>0.76-0.83 | 0.30<br>0.18-0.48 | 0.14<br>0.10-0.18 | 0.07<br>0.05-0.09 | 0.06<br>0.04-0.09 |

TABLE 8. RECOVERY OF 1,2,4 - TRICHLOROBENZENE (AS $^{14}C$) IN MALE RATS*

SINGLE INTRAVENOUS DOSE OF 10 MG/KG

| | Per Cent Dose at Various Times Post-Treatment | | | | | | |
|---|---|---|---|---|---|---|---|
| | 3 | 6 | 12 | 24 | 48 | 72 | 96 |
| Urine | 6.0 | 31.1 | 56.0 | 78.5 | 79.4 | 89.4 | 83.2 |
| Feces | <0.1 | <0.1 | 0.1 | 5.4 | 13.5 | 10.1 | 12.4 |
| G-I Tract | 10.7 | 20.5 | 13.9 | 5.2 | 0.8 | 0.3 | 0.2 |
| Tissues | 8.9 | 10.5 | 5.2 | 1.7 | 0.8 | 0.4 | 0.4 |
| Total Recovery | 25.6 | 62.1 | 75.2 | 90.8 | 94.5 | 100.2 | 96.2 |

* 4 rats per time period.

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## III. PHARMACOKINETIC STUDY OF TCB-$^{14}$C IN RHESUS MONKEYS

TCB-$^{14}$C has been administered in single 10 mg/kg doses to 4 rhesus monkeys; 2 received the compound intravenous and 2 orally. The purpose of this study was to collect range-finding data on the pharmacokinetics of TCB in rhesus monkeys, data to be used in designing the subchronic studies to follow.

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### Materials and Methods

The treatment solution was prepared by dissolving 220 µl (320 mg) of 1,2,4-trichlorobenzene-$^{14}$C (0.56 µCi/ml) and 124 µl (180 mg) of unlabeled TCB (Eastman Kodak Lot #1641) in 10 ml of Emulphor®:ethanol (1:1,v/v) and diluting this to a total volume of 50 ml with distilled $H_2O$. This solution contained 10 mg TCB and 2.14 µCi $^{14}$C per ml.

Adult female rhesus monkeys were tranquilized with ketamine HCl (4.5 mg/kg) and placed in restraining chairs three days prior to the administration of the compound. There was no apparent reaction to either intravenous or oral treatments with the compound. Blood samples were taken before treatment and at 1,2,4,6,8,12,24,48,72 and 96 hours post-treatment. Urine samples were collected at 6,12,24,48,72 and 96 hours post-treatment; feces were collected at 24 hour intervals for seven days.

Hematocrit was determined on each blood sample, and whole blood, plasma, urine, and feces samples were analyzed for total $^{14}$C content employing the standard procedures used in this laboratory.

### Results

Data on the blood and plasma levels of intravenous and orally treated animals are contained in Table 9. When TCB-$^{14}$C was administered intravenous there was a dramatic drop in $^{14}$C content of whole blood from an estimated zero time level of about 112 µg/ml to a level of 2.0-2.9 µg/ml one hour post-treatment. From this point on there was a slow decline over the next four days to levels of 0.2 µg/ml. The plasma concentrations of TCB-$^{14}$C were higher than the respective whole blood concentrations ranging from 3.1 to 4.9 µg/ml at one hour and declining biphasically to 0.24 µg/ml at 96 hours.

Rhesus monkey No. 3 which received the compound orally, exhibited a peak blood level of 3 µg/ml (4.6 µg/ml plasma) at one hour. Rhesus monkey No. 4, also orally treated, had a peak blood level of 2.3 µg/ml (3.6 µg/ml plasma) at two hours post-treatment. During the remaining period of observation, there were no significant differences in the blood or plasma levels of the intravenous and orally treated animals.

Urinary excretion following intravenous administration accounted for about 38% of the dose in four days; 22% was excreted during the first 24 hours (see Table 10). In the monkeys receiving TCB orally, urinary excretion accounted for a significantly greater amount of the compound; 36 to 40% was recovered during the first 24 hours and 56 to 73% of the dose in four days (Table 11).

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Figure 1 shows the dramatic difference between the urinary excretion of TCB following single oral or intravenous doses. This difference in urinary excretion between the animals receiving this compound intravenous and orally may be due to the fact that the parent, TCB, is very lipid soluble and is rapidly deposited in the fat depots following intravenous dosing. It is then slowly mobilized and metabolized by the liver. When the compound is administered orally, it is transported first to the liver where it can be stored and rapidly metabolized before entering the systemic circulation. Thus, after an oral dose, less of the compound is available for deposition in the body fat. This explanation is borne out by our findings in rats given TCB intravenous and orally. We found the concentration of this drug in fat was extremely high at three hours in intravenous animals (52 μg/g) while rats receiving the compound orally had only about one-third as much or 17 μg/g fat.

Fecal excretion of this compound by rhesus monkeys following either oral or intravenous treatment is insignificant, accounting for less than 4% of the total dose. This suggests that the compound is readily and completely absorbed from the gastrointestinal tract and quite readily metabolized to products easily excreted via the urine. The small amount of fecal radioactivity following intravenous treatment provides indirect evidence that there is less enterohepatic circulation of TCB and metabolites in the monkey than in the rat. Furthermore, TCB appears to be excreted somewhat more slowly by the monkey than by the rat.

## IV. FIRST SUBCHRONIC TOXICITY STUDY OF TCB IN MONKEYS. INTRODUCTION AND PRELIMINARY FINDINGS

The rather prolonged $T_{1/2}$ of the $\beta$ phase of TCB excretion observed in the simian pharmacokinetic studies suggested that this compound might accumulate in the body following repeated doses in monkeys. Data from other animal studies either suggested cumulative effects, or were inconclusive on this point. As a pilot, we administered repeated 50 mg/kg oral doses to one rhesus monkey and its responses suggested that this dose administered daily would probably prove fatal to monkeys in a relatively short period. For this reason we chose for our first subchronic study daily oral dose levels of 1,5 and 25 mg/kg.

### Materials and Methods

A. Animals. Twenty healthy, young adult female rhesus monkeys, previously used only as breeders, were obtained from Litton Bionetics. They ranged in weight from 5 to 8 kg and were housed either individually or with one compatible partner in large cages at Christ Hospital Institute of Medical Research, Cincinnati, Ohio, where the study was conducted. They were acclimated to their quarters and to a single experienced animal technician several months before the experiments were initiated. They were caught daily without being tranquilized, weighed at weekly intervals, and examined routinely. They were fed Purina Monkey Chow and water *ad libitum*.

TABLE 9. BLOOD AND PLASMA CONCENTRATION OF 1,2,4 - TRICHLOROBENZENE (AS $^{14}C$) IN FEMALE RHESUS MONKEYS

| | Single Intravenous Dose of 10 mg/kg | | | | | | Single Oral Dose of 10 mg/kg | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. 1 | | | No. 2 | | | No. 3 | | | No. 4 | | |
| Time Post Treatment | % Hct | Blood | Plasma | % Hct | Blood | Plasma | % Hct | Blood | Plasma | % Hct | Blood | Plasma |
| Hours | | | | | | | | | | | | |
| 1 | 45 | 2.9 | 4.9 | 41 | 2.0 | 3.1 | 36 | 3.0 | 4.6 | 42 | 1.5 | 2.5 |
| 2 | 44 | 2.6 | 4.5 | - | 1.5 | 2.3 | 36 | 2.9 | 4.5 | 40 | 2.3 | 3.6 |
| 4 | 43 | 1.8 | 3.0 | 41 | 1.2 | 1.7 | 34 | 2.0 | 2.9 | 39 | 1.3 | 2.3 |
| 6 | 42 | 1.3 | 2.3 | - | 0.67 | NS | 36 | 1.8 | 2.7 | 39 | 1.5 | 2.5 |
| 8 | 41 | 1.2 | 2.1 | - | 0.79 | NS | 36 | 1.4 | 2.2 | 41 | 1.1 | 1.8 |
| 12 | 41 | 0.8 | 1.4 | 38 | 0.48 | 0.75 | 32 | 0.94 | 1.4 | 39 | 0.67 | 1.0 |
| 24 | 40 | 0.49 | 0.76 | 40 | 0.35 | 0.53 | 32 | 0.54 | 0.79 | 37 | 0.43 | 0.60 |
| 48 | 37 | 0.35 | 0.54 | 36 | 0.26 | 0.33 | 32 | 0.29 | 0.36 | 37 | 0.27 | 0.38 |
| 72 | 35 | 0.23 | 0.36 | -[a] | - | - | 32 | 0.19 | 0.25 | 38 | 0.22 | 0.30 |
| 96 | 34 | 0.18 | 0.24 | - | - | - | 31 | 0.16 | 0.19 | 39 | 0.17 | 0.21 |

[a] Rhesus monkey No. 2 developed a detached seat-pad and was returned to cage at 56 hours post treatment.

## TABLE 10. CUMULATIVE EXCRETION OF 1,2,4 - TRICHLOROBENZENE (AS $^{14}C$) IN FEMALE RHESUS MONKEYS

SINGLE INTRAVENOUS DOSE OF 10 MG/KG

No. 1

| | Urine | | Feces | | Urine and Feces | |
|---|---|---|---|---|---|---|
| Hours | Period | Total | Period | Total | Period | Total |
| 0-24 | 22[a] | 22 | NS[b] | - | 22 | 22 |
| 24-48 | 5.4 | 27.4 | <0.01 | - | 5.4 | 27.4 |
| 48-72 | 6.2 | 33.6 | 0.1 | 0.1 | 6.3 | 33.7 |
| 72-96 | 4.7 | 38.3 | 1.7 | 1.8 | 6.4 | 40.1 |
| 96-120 | - | - | 1.1 | 2.9 | 1.1 | 41.2 |
| 120-144 | - | - | 0.5 | 3.4 | 0.5 | 41.7 |
| 144-168 | - | - | 0.4 | 3.8 | 0.4 | 42.1 |

No. 2

| | Urine | | Feces | | Urine and Feces | |
|---|---|---|---|---|---|---|
| Hours | Period | Total | Period | Total | Period | Total |
| 0-24 | 21.8 | 21.8 | NS | - | 21.8 | 21.8 |
| 24-48 | 6.3 | 28.0 | NS | - | 6.3 | 28.0 |
| 48-72 | 1.3 | 29.3 | NS | - | 1.3 | 29.3 |
| 72-96 | -[c] | - | 0.4 | 0.4 | 0.4 | 29.7 |
| 96-120 | - | - | 0.9 | 1.3 | 0.9 | 30.6 |
| 120-144 | - | - | 0.2 | 1.5 | 0.2 | 30.8 |
| 144-168 | - | - | 0.3 | 1.8 | 0.3 | 31.1 |

[a] Approximately 75% of total sample lost.

[b] NS- no sample.

[c] Rhesus monkey No. 2 developed a detached seat-pad and was returned to cage at 56 hrs post treatment.

## TABLE 11. CUMULATIVE EXCRETION OF 1,2,4 - TRICHLOROBENZENE (AS $^{14}$C) IN FEMALE RHESUS MONKEYS

SINGLE ORAL DOSE OF 10 MG/KG

No. 3

| | Urine | | Feces | | Urine and Feces | |
|---|---|---|---|---|---|---|
| Hours | Period | Total | Period | Total | Period | Total |
| 0-24 | 39.8 | 39.8 | <0.1 | - | 39.8 | 39.8 |
| 24-48 | 25.6 | 65.4 | 0.9 | 0.9 | 26.5 | 66.3 |
| 48-72 | 4.5 | 69.9 | 2.0 | 2.9 | 6.5 | 72.8 |
| 72-96 | 3.2 | 73.1 | 0.8 | 3.7 | 4.0 | 76.8 |
| 96-120 | -[a] | - | 0.1 | 3.8 | 0.1 | 76.9 |
| 120-144 | - | - | 0.2 | 4.0 | 0.2 | 77.1 |
| 144-168 | - | - | 0.1 | 4.1 | 0.1 | 77.2 |

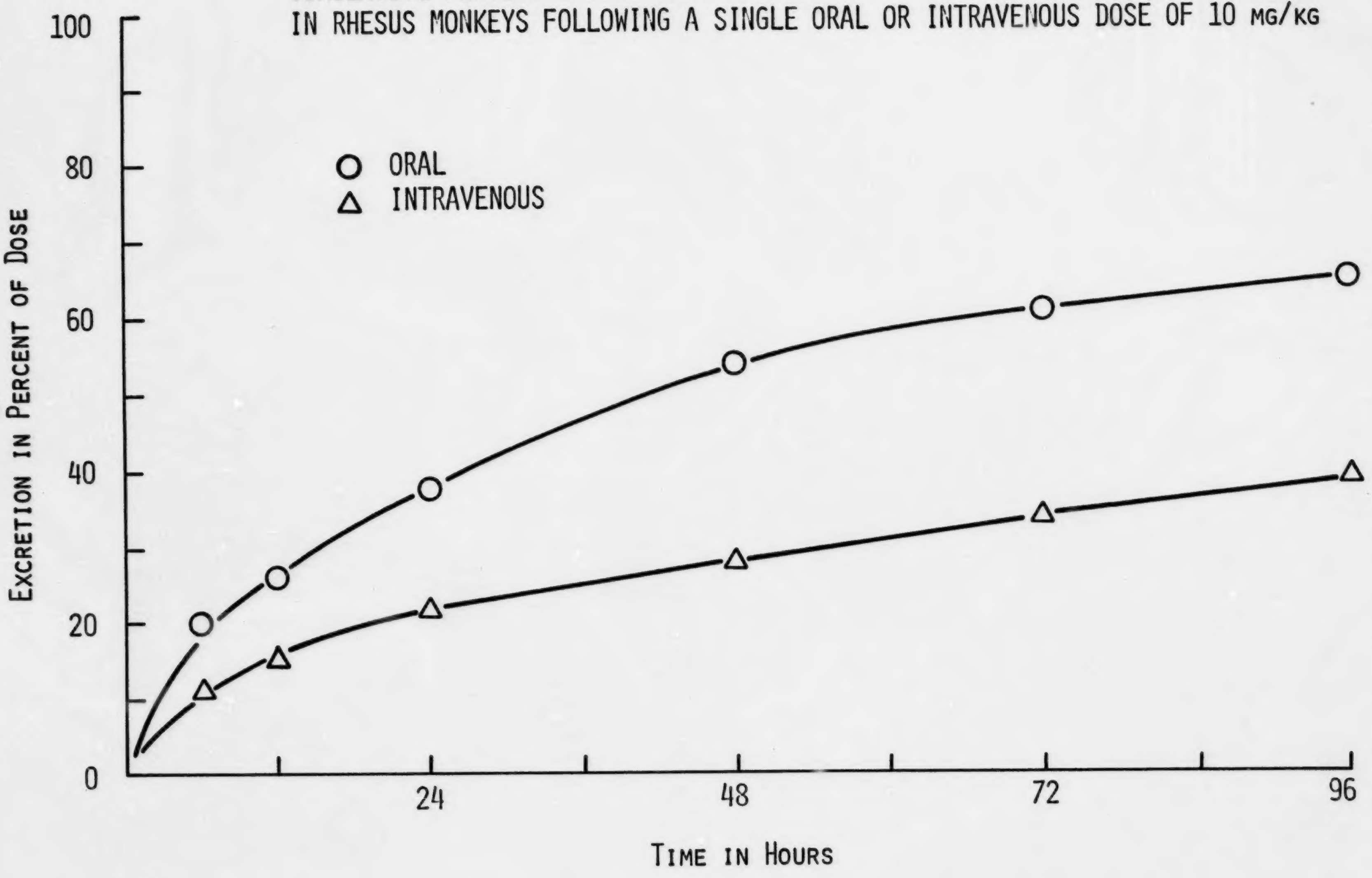
No. 4

| | Urine | | Feces | | Urine and Feces | |
|---|---|---|---|---|---|---|
| Hours | Period | Total | Period | Total | Period | Total |
| 0-24 | 36.7 | 36.7 | 0.1 | 0.1 | 36.8 | 36.8 |
| 24-48 | 10.1 | 46.8 | 0.1 | 0.2 | 10.2 | 47.0 |
| 48-72 | 5.7 | 52.5 | <0.1 | 0.2 | 5.7 | 52.7 |
| 72-96 | 4.2 | 56.7 | 0.5 | 0.7 | 4.7 | 57.4 |
| 96-120 | -[a] | - | 0.8 | 1.5 | 0.8 | 58.2 |
| 120-144 | - | - | 0.6 | 2.1 | 0.6 | 58.8 |
| 144-168 | - | - | 0.6 | 2.7 | 0.6 | 59.4 |

[a] Animal returned to cage--no further urine collections made.

FIGURE 1

CUMULATIVE PERCENT EXCRETION IN URINE OF 1,2,4 - TRICHLOROBENZENE (AS $^{14}C$) IN RHESUS MONKEYS FOLLOWING A SINGLE ORAL OR INTRAVENOUS DOSE OF 10 MG/KG



When urine and feces collections were required, the animals were placed in individual metabolism cages specially fabricated for primate metabolism studies. During these periods, urine was collected in iced containers and the portion not analyzed immediately was stored at -20°C.

Monkeys were gavaged with TCB each morning, seven days per week for three months. Treatments occurred at the same time each morning and required no more than 45 minutes. Each week when the monkeys were weighed, a blood sample was drawn for TCB metabolite analyses. Once monthly, additional blood was taken for SMA 12/60 clinical chemistry analyses. Chlorguanide (CG) metabolite profile tests were performed at 30-day intervals after initiation of TCB treatment.

Liver biopsies for enzyme analyses were obtained at the end of the three month treatment period through the generous cooperation of the staff veternarians in Dr. Steele Mattingly's Department of Laboratory Animal Medicine, University of Cincinnati, College of Medicine. The animals all recovered without sequelae in five days from this procedure.

B. Chemicals. 1,2,4-trichlorobenzene (TCB), was obtained from Aldrich Chemical Co., and most of the chemicals used in the in vitro MFO assays were obtained from Fisher, Sigma or Aldrich. One gram of benzphetamine hydrochloride was procured directly from the UpJohn Co., Kalamazoo, Michigan.

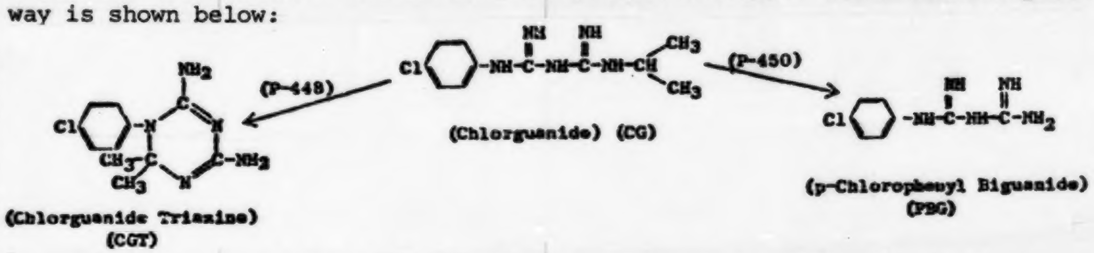
Chlorguanide hydrochloride (CG·HCl) was obtained from the DuPont Chemical Co. This compound was recrystallized to a melting point range of less than 1°C and an extinction coefficient at 258 nm of 712 in methanol. Radiolabeled CG·HCl was purchased from New England Nuclear, Inc. with $^{14}C$ at position 1 or 2 of the phenyl ring. This material had a very narrow melting point range and showed no radiolabeled contamination when chromatographed on cellulose TLC plates in three separate solvent systems. Chlorguanide metabolites, chlorguanide triazine (CGT) and p-chlorophenyl biguanide (PBG), were obtained from Parke-Davis, Inc.

C. Treatment Solutions. TCB was administered orally in a vehicle of Emulphor®:ethanol:$H_2O$ (1:1:38), similar to that used previously but more dilute. Treatment solutions were prepared so that the required dose/kg body weight was contained in 4 ml of solution.

CG·HCl was dissolved in distilled water at a concentration of 2.5 mg/ml. $^{14}C$-labeled CG·HCl was added to produce a minimal specific activity in the range of 175,000 dpm/ml; the actual specific activity for each treatment solution was determined by scintillation counting. These solutions were prepared fresh one day prior to each CG test and administered orally in a single dose of 10 mg/kg.

D. Chlorguanide Metabolite Profile Test. This is a new test, developed in this laboratory which is based on the principle that the activity of the mixed function oxidases (MFO) in the liver (the P-450 system) can be estimated on the basis of the profile of urinary metabolites of a compound metabolized primarily by this system. Ideally, the compound selected for this purpose should be metabolized to discrete products by each of the two major types of MFO, P-450 and P-448. In this way, the urinary concentrations of each metabolic product can be used to estimate the relative activities of P-450 and P-448 in the liver. Furthermore, the compound should normally be excreted in part unchanged so that one could measure the relative activity of each monoxygenase component present by comparing MFO-specific metabolites to parent compound.

One compound meeting these criteria is the antimalarial, chlorguanide (CG). It is oxidized by P-448 to the active antimalarial metabolite, chlorguanide triazine (CGT), and by P-450 to the inactive product, p-chlorophenyl biguanide (PBG) (5,6). Parent CG is also excreted (6). The metabolic pathway is shown below:



To determine its metabolic profile in urine we have developed colorimetric and microbiological methods for measuring CG, CGT, PBG (after conversion to CGT) and total CG metabolites. These procedures have been used in estimating CG and its principal metabolites CGT and PBG in blood, plasma, and urine ( 6 ). More recently, when radiolabeled CG became available, we developed a method for separating CG and its metabolites using TLC (S.T. Cragg, Doctoral Thesis, University of Cincinnati, 1980) and this latter procedure was the one employed principally in the studies reported here. Essentially the method is based on the extraction and separation of CG, CGT and PBG from a two day urine pool following CG dosing. An appropriate amount of the extract is spotted on a TLC plate and after development, the radioactivity in spots corresponding to the location of the 3 CG metabolic products is determined by LSC. The method has been shown to be reproducible and as will be demonstrated subsequently appears to be predictive of the relative activities of the "P-448" and "P-450" components of the MFO. Thus, if the "P-450" component is induced we would expect more PBG in the urine and correspondingly less CG and/or CGT. A "P-448" inducer like 3-MC should increase CGT formation and excretion at the expense of CG and/or PBG while an inducer like the PCB Aroclor 1254 should increase the formation of both CGT and PBG with a concurrent reduction in urinary CG.

## Results

The monkeys, which were treated with doses of TCB of 1,5, and 25 mg/kg for 3 months, showed neither weight loss, changes in clinical chemistry analyses (SMA 12/60) Tables 12 and 13, nor significant changes in their urinary CG metabolite profiles. Furthermore, there were no significant differences in the $T_{1/2}$ values for labeled TCB (as $^{14}C$) when a single intravenous dose was administered to the control and 25 mg/kg (TCB) groups at the end of 3 months treatment. For the sake of brevity these data are not included but can be made available to anyone upon request.

## TABLE 12. SMA 12/60 RESULTS ON MONKEYS BEFORE TREATMENT WITH TCB

| Dose mg/kg | Monkey No. | Sugar mg % | Uric acid mg % | Chol mg % | SGOT mU/ml | LDH mU/ml | P mg % | Alk.P. mU/ml | Ca mg % | Direct Bili-rubin mg % | Total Bili-rubin mg % | Pro-tein g % | Alb g % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 573 | 89 | 0.7 | 165 | 47 | 470 | 4.7 | 183 | 9.4 | 0.1 | 0.2 | 8.0 | 3.6 |
| | 483 | 76 | 0.7 | 180 | 25 | 195 | 3.6 | 81 | 10.2 | 0.1 | 0.3 | 7.7 | 3.8 |
| | 366 | 94 | 0.7 | 186 | 35 | 385 | 2.8 | 252 | 9.5 | 0.1 | 0.3 | 7.4 | 3.9 |
| | 495 | 74 | 0.8 | 192 | 26 | 300 | 2.1 | 195 | 9.5 | 0.1 | 0.3 | 7.2 | 3.7 |
| 1 | 543 | 85 | 0.9 | 170 | 31 | 255 | 3.2 | 75 | 9.3 | 0.1 | 0.3 | 8.5 | 3.5 |
| | 228 | 109 | 0.6 | 146 | 37 | >600 | 2.4 | 252 | 8.1 | 0.2 | 0.5 | 6.4 | 2.6 |
| | 264 | 105 | 0.7 | 124 | 25 | 364 | 4.3 | >350 | 9.9 | 0.1 | 0.2 | 8.1 | 2.7 |
| | 604 | 81 | 0.3 | 148 | 56 | 250 | 2.8 | 159 | 10.3 | 0.0 | 0.1 | 8.6 | 4.3 |
| 5 | 634 | 100 | 0.3 | 115 | 32 | 257 | 5.6 | 189 | 11.0 | 0.0 | 0.1 | 8.5 | 3.8 |
| | 505 | 95 | 0.8 | 195 | 21 | 249 | 4.3 | 120 | 10.4 | 0.1 | 0.3 | 8.2 | 3.9 |
| | 287 | 114 | 0.7 | 187 | 33 | 166 | 3.4 | 62 | 9.7 | 0.1 | 0.3 | 7.9 | 4.2 |
| | 39 | 66 | 0.8 | 180 | 31 | 290 | 4.1 | 223 | 9.9 | 0.1 | 0.3 | 8.1 | 3.5 |
| 25 | 343 | 80 | 0.8 | 195 | 18 | 196 | 4.4 | 105 | 9.1 | 0.2 | 0.4 | 8.3 | 3.6 |
| | 110 | 93 | 0.7 | 201 | 32 | 223 | 3.4 | 118 | 9.1 | 0.1 | 0.3 | 9.1 | 3.9 |
| | 500 | 80 | 1.0 | 225 | 20 | 237 | 5.1 | 83 | 9.3 | 0.2 | 0.3 | 8.4 | 4.0 |
| | 276 | 56 | 0.6 | 174 | 17 | 171 | 3.0 | 134 | 9.2 | 0.1 | 0.2 | 7.5 | 3.3 |
| 125 | C-12 | 115 | 0.3 | 145 | 40 | 225 | 3.3 | 225 | 10.7 | 0.0 | 0.1 | 7.8 | 4.3 |
| | C-14 | 100 | 0.2 | 160 | 17 | 170 | 2.6 | 91 | 10.1 | 0.0 | 0.2 | 8.2 | 4.0 |
| | C-18 | 85 | 0.3 | 155 | 35 | 245 | 2.8 | 274 | 10.9 | 0.0 | 0.2 | 8.0 | 4.6 |
| | 253 | 83 | 0.2 | 160 | 30 | 175 | 3.3 | 154 | 10.0 | 0.0 | 0.2 | 8.3 | 4.2 |
| Baseline Values | | 89 | 0.61 | 170.2 | 30.4 | 271.2 | 3.56 | 166.3 | 9.78 | 0.12 | 0.26 | 8.01 | 3.77 |
| S.E. | | ±3.5 | ±0.05 | ± 6.0 | ±2.2 | ±24.6 | ±0.21 | ±17.5 | ±0.16 | ±0.01 | ±0.02 | ±0.13 | ±0.11 |

Monkeys 573-276 assayed 12/17/76.
Monkeys C12-253 assayed 6/24/77.

## TABLE 13. SMA 12/60 RESULTS ON MONKEYS AFTER TREATMENT WITH TCB FOR TWO MONTHS

| Dose mg/kg | Monkey No. | Sugar mg % | Uric acid mg % | Chol mg % | SGOT mU/ml | LDH mU/ml | P mg % | Alk.P. mU/ml | Ca mg % | Direct Bili-rubin mg % | Total Bili-rubin mg % | Pro-tein g % | Alb g % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 573 | 77 | 0.3 | 150 | 31 | 295 | 3.6 | 255 | 8.8 | 0.0 | 0.2 | 8.0 | 3.5 |
|  | 483 | 81 | 0.2 | 151 | 24 | 226 | 3.1 | 137 | 9.6 | 0.0 | 0.2 | 7.9 | 3.8 |
|  | 366 | 85 | 0.2 | 175 | 25 | 255 | 2.5 | 206 | 9.4 | 0.0 | 0.2 | 7.6 | 4.0 |
|  | 495 | 82 | 0.2 | 162 | 19 | 215 | 2.3 | 217 | 9.2 | 0.0 | 0.2 | 7.6 | 4.1 |
| 1 | 543 | 80 | 0.3 | 175 | 29 | 255 | 4.2 | 96 | 9.4 | 0.0 | 0.2 | 8.9 | 4.1 |
|  | 228 | 85 | 0.2 | 205 | 20 | 249 | 3.8 | 227 | 9.5 | 0.0 | 0.2 | 8.1 | 4.2 |
|  | 264 | 110 | 0.2 | 150 | 20 | 210 | 3.7 | 324 | 9.3 | 0.0 | 0.2 | 8.6 | 3.8 |
|  | 604 | 95 | 0.3 | 150 | 42 | 335 | 4.4 | 170 | 10.0 | 0.0 | 0.3 | 8.9 | 4.6 |
| 5 | 634 | 84 | 0.2 | 125 | 17 | 232 | 3.3 | 185 | 9.9 | 0.0 | 0.1 | 7.8 | 4.5 |
|  | 505 | 85 | 0.2 | 190 | 19 | 360 | 2.2 | 178 | 10.4 | 0.0 | 0.1 | 8.1 | 4.9 |
|  | 287 | 78 | 0.2 | 198 | 23 | 255 | 2.4 | 95 | 10.3 | 0.0 | 0.1 | 8.4 | 4.4 |
|  | 39 | 90 | 0.2 | 160 | 25 | 335 | 2.5 | 144 | 9.6 | 0.0 | 0.1 | 8.4 | 3.8 |
| 25 | 343 | 63 | 0.3 | 188 | 17 | 223 | 4.2 | 131 | 9.0 | 0.0 | 0.2 | 8.4 | 4.5 |
|  | 110 | 97 | 0.1 | 200 | 20 | 189 | 3.2 | 117 | 9.8 | 0.0 | 0.2 | 9.0 | 4.0 |
|  | 500 | 88 | 0.3 | 200 | 18 | 245 | 4.9 | 140 | 10.3 | 0.0 | 0.2 | 9.2 | 5.2 |
|  | 276 | 55 | 0.1 | 205 | 20 | 210 | 4.3 | 188 | 9.8 | 0.0 | 0.1 | 9.0 | 4.3 |
| 125 | C-12 | 85 | 1.1 | 142 | 9 | 155 | 4.7 | 247 | 9.6 | 0.1 | 0.1 | 7.8 | 4.1 |
|  | C-14 | 77 | 1.0 | 145 | 5 | 123 | 3.5 | 159 | 9.7 | 0.0 | 0.0 | 8.5 | 4.2 |
|  | C-18 | 73 | 1.0 | 168 | 16 | 195 | 2.9 | 167 | 10.6 | 0.0 | 0.0 | 8.6 | 4.7 |
| Baseline Values |  | 87.3 | 0.69 | 173.9 | 30.4 | 288.0 | 3.70 | 161.3 | 9.62 | 0.12 | 0.28 | 7.99 | 3.64 |
| S.E. |  | ±3.9 | ±0.05 | ± 7.2 | ±2.6 | ±29.1 | ±0.24 | ±20.0 | ±0.17 | ±0.01 | ±0.03 | ±0.16 | ±0.12 |

## V. SECOND TCB SUBCHRONIC STUDY

After the first TCB study had been in progress about one month it became obvious that none of the animals were showing toxic symptoms and that one of the important objectives of the study, an estimation of the chronic maximum tolerated dose of TCB, was not likely to be accomplished. Therefore, we added another geometrically-spaced dose level, 125 mg/kg, which we expected to be toxic. After one month it again became clear that although two monkeys from the 125 mg/kg group showed temporary weight loss and one animal died in 16 days, the three remaining animals appeared healthy, began to gain weight, and showed no signs or symptoms of toxicity specifically related to TCB.

We had demonstrated to our satisfaction that the 1,5 and 25 mg/kg TCB-dosed monkeys in the first subchronic study showed no evidence whatever of changes related to TCB administration. This decision was based on the weight curves, SMA 12/60 analyses, CG profiles, T½ for intravenous TCB and biopsy findings. Therefore, we assigned half of each 5 and 25 mg/kg group to two new dose levels - 90 and 174 mg/kg - dose levels chosen to be logarithmically spaced above and below the 125 mg/kg group. These three groups of monkeys as well as the original control group comprise the Second TCB Study.

The treatment solutions were exactly like those used in the first subchronic study except that the concentration of Emulphor® in the medium had to be increased to 10% to keep the larger amounts of TCB in solution. The control group also received this revised menstruum.

The general protocol was the same as we used before. The animals were treated orally 7 days/week. They were weighed at weekly intervals and blood samples were collected at the same time. Once monthly the animals were placed in metabolism cages and the urinary profiles of chlorguanide (CG) metabolites were measured. Also at monthly intervals blood samples were collected for SMA 12/60 clinical chemistry analyses. At the end of 12 weeks, or at death, the animals were necropsied and liver slices were frozen in liquid $N_2$ for P-450 related enzyme assays.

### General Toxicity of TCB

The monkeys receiving these higher doses of TCB exhibited definite dose-related evidence of toxicity. The one animal on the 90 mg/kg dose that died in the 7th week (47 d) lost 0.7 kg. However, the lack of definitive toxic manifestations before death and at necropsy suggested that the animal may have succumbed to unknown but probably nondrug related causes. It did show a sizable increase in liver wt/body wt ratio and some definite changes in CG profile (Table 14). The weight changes of this group were very interesting and suggested that the animals were showing their greatest response to TCB at 4-6 weeks; thereafter the animals improved.

The changes in response to the 125 mg/kg dose have been mentioned above. Again we note that the maximum response to TCB appears to come after 4-6 weeks treatment after which the animals either became stable or improved.

The 174 mg/kg dose was definitely lethal and would have killed all four monkeys in this group had we not discontinued treatment after day 25. At this time the two surviving monkeys showed symptoms of extreme intoxication including weight loss, weakness, tremors, and increased susceptibility to pulmonary infection.

Recovery from the visibly toxic effects of TCB was rapid after treatment was discontinued. Although one additional monkey died after treatment was discontinued, this animal (#343) appeared to be recovering but apparently succumbed to acute gastric dilatation, probably the result of overeating due to careless overfeeding.

The weight changes in the monkeys treated with large doses of TCB are shown in Table 14. The controls showed no significant weight changes during the 10 week period shown in the table. For the 90 and 125 mg/kg groups the responses were quite variable. Two monkeys on the 90 mg/kg lost 1 and 2 kg, respectively. A third monkey did not change weight significantly. The fourth animal died but lost weight primarily in the last week of treatment. This death we believe may not be due to TCB. In the case of the 125 mg/kg dose the monkey that died (#253) lost 0.9 kg in two weeks and died on day 16. The other two monkeys (C18 and C14) showed early large weight losses (0.8 and 0.9 kg) but then became stable and at the end they,and C12, were gaining weight.

It would appear likely that if the animal responds to TCB by developing hepatic MFO induction, then the liver will be able to better handle the compound and from this point on the increased metabolism will reduce the effective dose.

Changes in hematocrit are shown in Table 15 and in general one sees the same trends observed with the changes in weight.TCB treatment tends to cause a decline in the packed red cell volume. We have not made any attempt to examine the underlying causes of this phenomenon.

The changes in liver to body weight ratios (as %) are shown in Tables 16 and 17. For the 90 mg/kg dose, the only highly significant change was seen in monkey #110 which died after 47 days of treatment. The percentages for the other three monkeys were scarcely above the normal range. All the monkeys receiving 125 and 174 mg/kg doses of TCB had liver wt/body wt ratios significantly higher than the controls. However, the ratio for #39 which had been off treatment for one month shows considerable recovery; the ratio now was 2.7%, one slightly higher than the mean for the controls (2.25%).

Data on the changes in CG metabolite profiles after 1,2 and 3 months of TCB treatment are shown in Tables 18, 19 and 20. The parameters measured included changes in % CG, PBG and CGT excreted in the urine as well as changes in the ratios of CGT/CG, PBG/CG and CGT/PBG. The changes observed after administration of TCB for one month are depicted graphically in Figure 2. The graphs indicate that TCB administration caused significant decrease in % CG in the urine, an increase in % PBG and no change in % CGT in the urine. The value of the ratios CGT/CG and PBG/CG was increased significantly by TCB administration while there was a significant decrease in the CGT/PBG ratio.

Comparison of the data after one month TCB treatment (Table 18 and Figure 2) with the findings after two and three months (Figures 3 and 4) suggests that administration of TCB led to an induction of MFO activity in the liver and that this increase appeared to be restricted to the P-450 class of MFO's. We found a decrease in CG excretion, an increase in PBG, and a decrease or no change in CGT excretion as well as changes in certain ratios, especially CGT/PBG.

At the termination of TCB administration the monkeys were necropsied, the livers were weighed, and slices of liver were stored in liquid $N_2$ for *in vitro* assay of MFO activity using several substrates. The results of these *in vitro* studies are described later.

TABLE 14. WEIGHTS OF MONKEYS ON DAILY TREATMENT WITH TCB

| Dose mg/kg | Monkey No. | Initial kg | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Time in Weeks<br>Weight in Kg | | | | | |
| 0 | 573 | 7.5 | 7.4 | 7.4 | 7.6 | 7.6 | 7.5 | 7.6 | 7.6 | 7.6 | 7.6 | 7.6 |
| | 483 | 6.0 | 5.9 | 5.9 | 6.2 | 6.3 | 6.2 | 6.2 | 6.4 | 6.4 | 6.4 | 6.5 |
| | 366 | 5.5 | 5.5 | 5.5 | 5.6 | 5.7 | 5.6 | 5.6 | 5.6 | 5.5 | 5.5 | 5.6 |
| | 495 | 5.6 | 5.5 | 5.6 | 5.6 | 5.7 | 5.6 | 5.7 | 5.9 | 5.8 | 5.8 | 5.7 |
| 90 | 634 | 7.8 | 7.0 | 6.4 | 5.6 | 5.3 | 5.6 | 5.2 | 5.1 | 5.4 | 5.6 | 5.8 |
| | 505 | 6.4 | 6.5 | 6.3 | 6.1 | 5.8 | 5.6 | 5.7 | 5.5 | 5.5 | 5.5 | 5.4 |
| | 110 | 5.9 | 5.9 | 5.8 | 5.7 | 5.6 | 5.5 | 5.2 | Died | | | |
| | 500 | 6.3 | 6.3 | 6.4 | 6.4 | 6.3 | 6.5 | 6.5 | 6.3 | 6.4 | 6.3 | 6.1 |
| 125 | C18 | 5.1 | 4.7 | 4.3 | 4.3 | 4.3 | 4.5 | 4.6 | 4.9 | 5.3 | 5.3 | 5.3 |
| | C14 | 6.3 | 5.8 | 5.4 | 5.6 | 5.8 | 6.1 | 6.6 | 6.6 | 6.7 | 6.7 | 6.7 |
| | C12 | 5.0 | 5.1 | 5.1 | 5.1 | 5.2 | 5.2 | 5.3 | 5.2 | 5.1 | 5.4 | 5.5 |
| | 253 | 5.3 | 5.1 | 4.4 | Died | | | | | | | |
| 174 | 287 | 6.2 | 5.8 | 5.5 | 5.0 | Died | | | | | | |
| | 39 | 6.3 | 5.9 | 5.2 | 4.5 | 4.2* | 4.3 | 4.8 | 5.2 | 5.6 | 5.6 | 5.8 |
| | 343 | 6.4 | 5.4 | 5.2 | 5.4 | 4.9* | 5.2 | Died+ | | | | |
| | 276 | 6.3 | 5.8 | 5.4 | 5.0 | Died | | | | | | |

*Dosing discontinued on day 25.

+Died of apparent overeating; the animal was exhibiting none of the severe toxic symptoms observed during the treatment period.

## TABLE 15. HEMATOCRITS OF MONKEYS ON DAILY TREATMENT WITH TCB

| Dose mg/kg | Monkey No. | Initial Hct | Time in Weeks 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 573 | 45 | - | 49 | 47 | 47 | 49 | 47 | 47 | 48 | 48 | 49 |
| | 483 | 43 | - | 52 | 52 | 50 | 53 | 51 | 49 | 54 | 52 | 52 |
| | 366 | 47 | - | 51 | 49 | 47 | 51 | 50 | 48 | 48 | 49 | 49 |
| | 495 | | - | 39 | 39 | 40 | 42 | 41 | 43 | 42 | 42 | 43 |
| 90 | 634 | 45 | 48 | 45 | 44 | 40 | 37 | 40 | 34 | 37 | 39 | 41 |
| | 505 | 47 | 46 | 44 | 44 | 44 | 42 | 41 | 39 | 40 | 40 | 41 |
| | 110 | 41 | 42 | 42 | 42 | 42 | 43 | 43 | (Died day 47) | | - | - |
| | 500 | 31 | 31 | 33 | 34 | 35 | 35 | 35 | 35 | 35 | 35 | 35 |
| 125 | C18 | 46 | 42 | 45 | 40 | 36 | 36 | 39 | 39 | 40 | 39 | 40 |
| | C14 | 44 | 41 | 41 | 36 | 39 | 37 | 34 | 34 | 40 | 38 | 37 |
| | C12 | 43 | 39 | 38 | 37 | 40 | 40 | 37 | 39 | 38 | 38 | 39 |
| | 253 | 48 | 40 | (Died day 16) | | | | | | | | |
| 174 | 287 | 41 | 42 | 40 | 39 | (Died day 28) | | - | - | - | - | - |
| | 39 | 46 | 46 | 46 | 43* | 40 | 38 | 39 | 41 | 43 | 44 | 45 |
| | 343 | 41 | 46 | 43 | 39* | 41 | 40 | (Died day 35 of overeating) | | | | - |
| | 276 | 44 | 44 | 44 | 42 | (Died day 24) | | - | - | - | - | - |

*Dosing discontinued on day 25.

TABLE 16. LIVER WEIGHT, BODY WEIGHT AND LIVER WEIGHT/BODY WEIGHTS RATIOS (AS PERCENTAGES) OF TCB TREATED MONKEYS AND CONTROLS

| Treatment | Monkey No. | Liver Wt (kg) | Initial Body Wt (kg) | Sacrifice Body Wt (kg) | Liver Wt x 100 / Init Body Wt | Liver Wt x 100 / Sac Body Wt |
|---|---|---|---|---|---|---|
| Emulphor Vehicle | 573 | 0.144 | 7.50 | 7.40 | 1.93 | 1.95 |
| | 483 | 0.150 | 6.00 | 6.50 | 2.51 | 2.32 |
| | 366 | 0.131 | 5.50 | 5.35 | 2.39 | 2.45 |
| | 495 | 0.132 | 5.60 | 5.80 | 2.37 | 2.28 |
| 90 mg/kg TCB | 634 | 0.171 | 7.80 | 6.10 | 2.20 | 2.81 |
| | 505 | 0.153 | 6.40 | 5.40 | 2.40 | 2.85 |
| | 110* | 0.219 | 5.90 | 4.70 | 3.73 | 4.68 |
| | 500 | 0.149 | 6.30 | 5.95 | 2.37 | 2.50 |
| 125 mg/kg TCB | C18 | 0.185 | 5.10 | 5.10 | 3.64 | 3.64 |
| | C14 | 0.218 | 6.30 | 5.90 | 3.46 | 3.70 |
| | C12 | 0.181 | 5.00 | 5.85 | 3.63 | 3.10 |
| | 253* | 0.214 | 5.30 | 4.40 | 4.04 | 4.86 |
| 174 mg/kg TCB | 287* | 0.218 | 6.20 | 4.70 | 3.53 | 4.66 |
| | 343 | 0.208 | 6.40 | 5.20 | 3.25 | 4.00 |
| | 39[a] | 0.164 | 6.30 | 6.10 | 2.62 | 2.70 |
| | 276*[b] | >0.135 | 6.30 | 4.50 | >2.1 | >3.0 |

*Monkeys which died during treatment.

[a] This subject had been withdrawn from treatment for over one month prior to sacrifice.

[b] Not all of this subject's liver was recovered.

TABLE 17. SIGNIFICANCE LEVELS (P) COMPARING LIVER WEIGHTS AND LIVER WT./BODY WT. PERCENTAGES AMONG CONTROL AND TCB TREATED GROUPS*

| Comparison | Liver Wts. | $\frac{\text{Liver Wt.}}{\text{Initial Body Wt.}} \times 100$ | $\frac{\text{Liver Wt.}}{\text{Sacrifice Body Wt.}} \times 100$ |
|---|---|---|---|
| "Overall" F | p = 0.0141 | 0.0091 | 0.0320 |
| ........................ Contrasts ........................ | | | |
| Control vs. Pooled TCB Treated Groups | 0.0031 | 0.0081 | 0.0065 |
| Dose-Response | 0.1037 | 0.1283 | 0.1071 |
| Curvilinearity | 0.8364 | 0.1192 | 0.8443 |
| Controls vs. 90's | 0.0607 | 0.2746 | 0.0938 |
| Controls vs. 125's | 0.0042 | 0.0020 | 0.0133 |
| Controls vs. 174's | 0.0115 | 0.0395 | 0.0157 |

*Significance levels were derived by a one-way ANOVA program capable of contrast evaluation.

TABLE 18. EFFECTS OF 30 DAYS TCB TREATMENT ON URINARY CHLORGUANIDE EXCRETION PARAMETERS

| TCB Dose mg/kg/day | Monkey No. | Percent CG B[a] | Percent CG Treated | Percent CG D[b] | Percent PBG B | Percent PBG Treated | Percent PBG D | Percent CGT B | Percent CGT Treated | Percent CGT D |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 573 | 10.1 | 13.9 | 3.8 | 37.5 | 38.4 | 0.9 | 52.4 | 47.8 | -4.6 |
| | 483 | 17.8 | 22.0 | 4.2 | 39.7 | 38.5 | -1.2 | 42.5 | 39.4 | -3.3 |
| | 366 | 17.7 | 17.0 | -0.7 | 36.6 | 37.5 | 0.9 | 45.7 | 45.4 | -0.3 |
| | 495 | 17.6 | 18.3 | 0.7 | 40.8 | 41.9 | 1.1 | 41.5 | 39.8 | -1.7 |
| | Mean | 15.8 | 17.8 | 2.0 | 38.7 | 39.1 | 0.4 | 45.5 | 43.1 | -2.5 |
| | SEM | ±1.90 | ±1.68 | ±1.2 | ±0.97 | ±0.97 | ±0.5 | ±2.46 | ±2.08 | ±0.94 |
| 90 | 634 | 12.0 | 12.2 | 0.2 | 36.9 | 47.2 | 10.3 | 51.1 | 40.6 | -10.5 |
| | 505 | 14.5 | 15.7 | 1.2 | 39.6 | 46.2 | 6.6 | 45.9 | 38.0 | -7.9 |
| | 110 | 11.5 | 9.96 | -1.5 | 41.6 | 46.0 | 4.4 | 48.9 | 44.2 | -4.7 |
| | 500 | 10.1 | 13.2 | 3.1 | 38.6 | 44.4 | 5.8 | 47.3 | 42.4 | -4.9 |
| | Mean | 12.0 | 12.8 | 0.7 | 39.2 | 45.9 | 6.8 | 48.3 | 41.3 | -7.0 |
| | SEM | ±0.92 | ±1.19 | ±1.0 | ±0.98 | ±0.58 | ±1.3 | ±1.12 | ±1.32 | ±1.4 |
| 125[c] | C18 | 15.4 | 9.80 | -5.6 | 39.0 | 54.1 | 15.1 | 45.7 | 36.1 | -9.6 |
| | C14 | 14.6 | 12.6 | -2.0 | 40.5 | 44.1 | 3.6 | 44.8 | 43.3 | -1.5 |
| | C12 | 15.2 | 10.5 | -4.7 | 43.4 | 50.0 | 6.6 | 41.4 | 39.5 | -1.9 |
| | Mean | 15.1 | 11.0 | -4.1 | 41.0 | 49.4 | 8.4 | 44.0 | 39.6 | -4.3 |
| | SEM | ±0.24 | ±0.84 | ±1.1 | ±1.29 | ±2.90 | ±3.4 | ±1.31 | ±2.08 | ±2.6 |
| 174[d] | 343 | 10.7 | 10.6 | -0.1 | 38.8 | 47.3 | 8.5 | 50.4 | 42.2 | -8.2 |
| | 39 | 14.1 | 11.0 | -3.1 | 38.6 | 49.0 | 10.4 | 47.3 | 39.9 | -7.4 |
| | Mean | 12.4 | 10.8 | -1.6 | 38.7 | 48.2 | 9.5 | 48.8 | 41.0 | -7.8 |

(continued)

TABLE 18 (continued)

| TCB Dose mg/kg/day | Monkey No. | CGT/CG B[a] | CGT/CG Treated | CGT/CG D[b] | PBG/CG B | PBG/CG Treated | PBG/CG D | CGT/PBG B | CGT/PBG Treated | CGT/PBG D |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 573 | 5.17 | 3.44 | -1.73 | 3.71 | 2.76 | -0.95 | 1.40 | 1.24 | -0.160 |
| | 483 | 2.38 | 1.78 | -0.60 | 2.22 | 1.75 | -0.47 | 1.07 | 1.02 | -0.050 |
| | 366 | 2.58 | 2.68 | 0.10 | 2.07 | 2.21 | 0.14 | 1.25 | 1.21 | -0.040 |
| | 495 | 2.35 | 2.18 | -0.17 | 2.31 | 2.29 | -0.02 | 1.02 | 0.956 | -0.064 |
| | Mean | 3.12 | 2.52 | -0.60 | 2.58 | 2.25 | -0.33 | 1.19 | 1.11 | -0.080 |
| | SEM | ±0.69 | ±0.36 | ±0.40 | ±0.38 | ±0.21 | ±0.25 | ±0.09 | ±0.07 | ±0.030 |
| 90 | 634 | 4.25 | 3.35 | -0.90 | 3.07 | 4.88 | 1.81 | 1.38 | 0.863 | -0.517 |
| | 505 | 3.16 | 2.45 | -0.71 | 2.73 | 2.96 | 0.23 | 1.16 | 0.824 | -0.336 |
| | 110 | 4.26 | 4.52 | 0.26 | 3.46 | 4.70 | 1.24 | 1.23 | 0.961 | -0.269 |
| | 500 | 4.68 | 3.38 | -1.30 | 4.22 | 3.49 | -0.73 | 1.11 | 0.959 | -0.151 |
| | Mean | 4.09 | 3.43 | -0.66 | 3.37 | 4.01 | 0.64 | 1.22 | 0.904 | -0.320 |
| | SEM | ±0.33 | ±0.42 | ±0.33 | ±0.32 | ±0.46 | ±0.56 | ±0.06 | ±0.030 | ±0.080 |
| 125[c] | C18 | 2.98 | 3.68 | 0.70 | 2.54 | 5.52 | 2.98 | 1.17 | 0.668 | -0.502 |
| | C14 | 3.07 | 3.42 | 0.35 | 2.77 | 3.48 | 0.71 | 1.11 | 0.982 | -0.128 |
| | C12 | 2.72 | 3.77 | 1.05 | 2.86 | 4.77 | 1.91 | 0.953 | 0.790 | -0.163 |
| | Mean | 2.92 | 3.62 | 0.70 | 2.72 | 4.59 | 1.87 | 1.08 | 0.812 | -0.264 |
| | SEM | ±0.10 | ±0.10 | ±0.20 | ±0.10 | ±0.60 | ±0.66 | ±0.06 | ±0.090 | ±0.120 |
| 174[d] | 343 | 4.72 | 4.10 | -0.62 | 3.64 | 4.59 | 0.95 | 1.30 | 0.890 | -0.410 |
| | 39 | 3.35 | 3.61 | 0.26 | 2.74 | 4.44 | 1.70 | 1.22 | 0.814 | -0.406 |
| | Mean | 4.04 | 3.86 | 0.18 | 3.19 | 4.52 | 1.33 | 1.26 | 0.850 | -0.408 |

[a] B = Baseline (pretreatment values).

[b] D (Delta) = 30 day treated values minus baselines.

[c] One monkey died before this CG test.

[d] Two monkeys died before this CG test.

TABLE 19. EFFECTS OF 60 DAYS TCB TREATMENT ON URINARY CHLORGUANIDE EXCRETION PARAMETERS

| TCB Dose mg/kg/day | Monkey No. | Percent CG B[a] | Percent CG Treated | Percent CG D[b] | Percent PBG B | Percent PBG Treated | Percent PBG D | Percent CGT B | Percent CGT Treated | Percent CGT D |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 573 | 10.1 | 14.4 | 4.3 | 37.5 | 38.6 | 1.1 | 52.4 | 47.2 | -5.2 |
| | 483 | 17.8 | 20.0 | 2.2 | 39.7 | 39.6 | -0.1 | 42.5 | 40.2 | -2.3 |
| | 366 | 17.7 | 14.6 | -3.1 | 36.6 | 38.9 | 2.3 | 45.7 | 46.4 | 0.7 |
| | 495 | 17.6 | 15.9 | -1.7 | 40.8 | 41.0 | 0.2 | 41.5 | 43.1 | 1.6 |
| | Mean | 15.8 | 16.2 | 0.4 | 38.7 | 39.5 | 0.9 | 45.5 | 44.2 | -1.3 |
| | SEM | ±1.90 | ±1.30 | ±1.7 | ±0.97 | ±0.53 | ±0.5 | ±2.46 | ±1.61 | ±1.5 |
| 90 | 634 | 12.0 | 13.8 | 1.8 | 36.9 | 44.8 | 7.9 | 51.1 | 41.4 | -9.7 |
| | 505 | 14.5 | 14.0 | -0.5 | 39.6 | 44.2 | 4.6 | 45.9 | 41.8 | -4.1 |
| | 500 | 10.1 | 13.0 | 0.9 | 38.6 | 43.1 | 4.5 | 47.3 | 43.8 | -3.5 |
| | Mean | 12.2 | 13.6 | 0.7 | 38.4 | 44.0 | 5.7 | 48.1 | 42.3 | -5.8 |
| | SEM | ±1.27 | ±0.30 | ±0.7 | ±0.79 | ±0.50 | ±1.1 | ±1.55 | ±0.74 | ±2.0 |
| 125[c] | C18 | 15.4 | 11.7 | -3.7 | 39.0 | 49.5 | 10.5 | 45.7 | 38.8 | -6.9 |
| | C14 | 14.6 | 19.8 | 5.2 | 40.5 | 40.8 | 0.3 | 44.8 | 39.4 | -5.4 |
| | C12 | 15.2 | 12.7 | -2.5 | 43.4 | 46.6 | 3.2 | 41.4 | 40.6 | -0.8 |
| | Mean | 15.1 | 14.7 | -0.3 | 41.0 | 45.6 | 4.7 | 44.0 | 39.6 | -4.4 |
| | SEM | ±0.24 | ±2.55 | ±2.8 | ±1.29 | ±2.56 | ±3.0 | ±1.31 | ±0.53 | ±1.8 |
| 174[d] | 39[e] | 14.1 | 11.2[e] | -2.9 | 38.6 | 41.3[e] | 2.7 | 47.3 | 47.5[e] | 0.2 |

(continued)

TABLE 19 (continued)

| TCB Dose mg/kg/day | Monkey No. | CGT/CG B[a] | CGT/CG Treated | CGT/CG D[b] | PBG/CG B | PBG/CG Treated | PBG/CG D | CGT/PBG B | CGT/PBG Treated | CGT/PBG D |
|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 573 | 5.17 | 3.32 | -1.85 | 3.71 | 2.71 | -1.00 | 1.40 | 1.22 | -0.180 |
| | 483 | 2.38 | 2.01 | -0.37 | 2.22 | 1.98 | -0.24 | 1.07 | 1.01 | -0.060 |
| | 366 | 2.58 | 3.18 | 0.60 | 2.07 | 2.67 | 0.60 | 1.25 | 1.20 | -0.050 |
| | 495 | 2.35 | 2.72 | 0.37 | 2.31 | 2.58 | 0.27 | 1.02 | 1.06 | 0.040 |
| | Mean | 3.12 | 2.81 | -0.31 | 2.58 | 2.49 | -0.09 | 1.19 | 1.12 | -0.060 |
| | SEM | ±0.69 | ±0.30 | ±0.55 | ±0.38 | ±0.17 | ±0.35 | ±0.09 | ±0.050 | ±0.050 |
| 90 | 634 | 4.25 | 2.99 | -1.26 | 3.07 | 3.24 | 0.17 | 1.38 | 0.924 | -0.456 |
| | 505 | 3.16 | 2.99 | -0.17 | 2.73 | 3.16 | 0.43 | 1.16 | 0.946 | -0.214 |
| | 500 | 4.68 | 3.36 | -1.32 | 4.22 | 3.30 | -0.92 | 1.11 | 1.02 | -0.090 |
| | Mean | 4.03 | 3.11 | -0.92 | 3.34 | 3.23 | -0.11 | 1.22 | 0.960 | -0.250 |
| | SEM | ±0.45 | ±0.12 | ±0.37 | ±0.45 | ±0.04 | ±0.41 | ±0.08 | ±0.030 | ±0.110 |
| 125[c] | C18 | 2.98 | 3.30 | 0.32 | 2.54 | 4.21 | 1.67 | 1.17 | 0.784 | -0.386 |
| | C14 | 3.07 | 1.99 | -1.08 | 2.77 | 2.07 | -0.70 | 1.11 | 0.965 | -0.145 |
| | C12 | 2.72 | 3.20 | 0.48 | 2.86 | 3.67 | 0.81 | 0.953 | 0.871 | -0.082 |
| | Mean | 2.92 | 2.83 | -0.09 | 2.72 | 3.32 | 0.59 | 1.08 | 0.870 | -0.204 |
| | SEM | ±0.10 | ±0.42 | ±0.50 | ±0.10 | ±0.64 | ±0.69 | ±0.06 | ±0.050 | ±0.090 |
| 174[d] | 39[e] | 3.35 | 4.26[e] | 0.91 | 2.74 | 3.71[e] | 0.97 | 1.22 | 1.14[e] | -0.080 |

[a] B = Baseline (pretreatment values).

[b] D (Delta) = 30 day treated values minus baselines.

[c] One monkey died before the first CG test.

[d] A third monkey from this group died between the first and second CG tests.

[e] Monkey 39 had been withdrawn from TCB treatment for over 1 month prior to this CG test.

TABLE 20. EFFECTS OF 90 DAYS TCB TREATMENT ON URINARY CHLORGUANIDE EXCRETION PARAMETERS

| TCB Dose mg/kg/day | Monkey No. | Percent CG B[a] | Percent CG Treated | Percent CG D[b] | Percent PBG B | Percent PBG Treated | Percent PBG D | Percent CGT B | Percent CGT Treated | Percent CGT D |
|---|---|---|---|---|---|---|---|---|---|---|
| 125[c] | C18 | 15.4 | 6.41 | -8.99 | 39.0 | 55.6 | 16.6 | 45.7 | 37.9 | -7.8 |
| | C14 | 14.6 | 18.4 | 3.80 | 40.5 | 43.8 | 3.3 | 44.8 | 37.7 | -7.1 |
| | C12 | 15.2 | 12.7 | -2.50 | 43.4 | 47.0 | 3.6 | 41.4 | 40.3 | -1.1 |
| | Mean | 15.1 | 12.5 | -2.56 | 41.0 | 48.8 | 7.8 | 44.0 | 38.6 | -5.3 |
| | SEM | ±0.24 | ±3.46 | ±3.69 | ±1.29 | ±3.52 | ±4.4 | ±1.31 | ±0.84 | ±2.1 |

(continued)

TABLE 20 (continued)

| TCB Dose | Monkey |  | CGT/CG |  |  | PBG/CG |  |  | CGT/PBG |  |
|---|---|---|---|---|---|---|---|---|---|---|
| mg/kg/day | No. | B[a] | Treated | D[b] | B | Treated | D | B | Treated | D |
| 125[c] | C18 | 2.98 | 5.92 | 2.94 | 2.54 | 8.68 | 6.14 | 1.17 | 0.682 | -0.488 |
|  | C14 | 3.07 | 2.05 | -1.02 | 2.77 | 2.38 | -0.39 | 1.11 | 0.860 | -0.250 |
|  | C12 | 2.72 | 3.18 | 0.46 | 2.86 | 3.71 | 0.85 | 0.953 | 0.859 | -0.094 |
|  | Mean | 2.92 | 3.72 | 0.79 | 2.72 | 4.92 | 2.20 | 1.08 | 0.800 | -0.280 |
|  | SEM | ±0.10 | ±1.15 | ±1.16 | ±0.10 | ±1.92 | ±2.00 | ±0.06 | ±0.060 | ±0.110 |

[a] B = Baseline (pretreatment values).

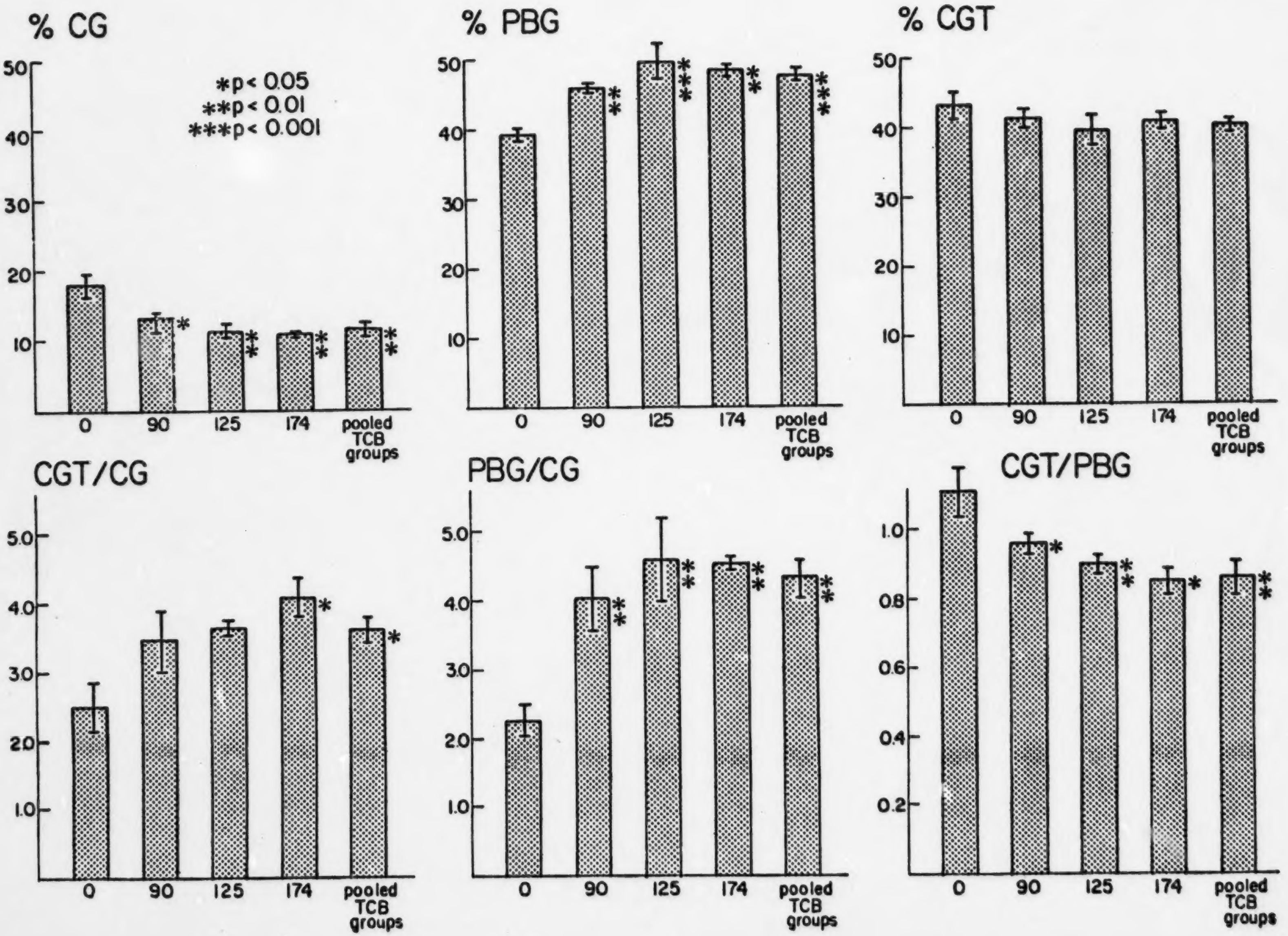
[b] D (Delta) = 30 day treated values minus baselines.

[c] One monkey died before the first CG test.

FIGURE 2

EFFECT OF 30 DAYS TCB PRETREATMENT ON URINARY CHLORGUANIDE EXCRETION PARAMETERS

( TCB treated monkeys compared to vehicle treated controls )



# FIGURE 3

EFFECT OF 30 DAYS TCB PRETREATMENT ON URINARY CHLORGUANIDE EXCRETION PARAMETERS

( TCB treated "Δ's" compared to control "Δ's", where Δ = post-treatment values minus pretreatment baselines )

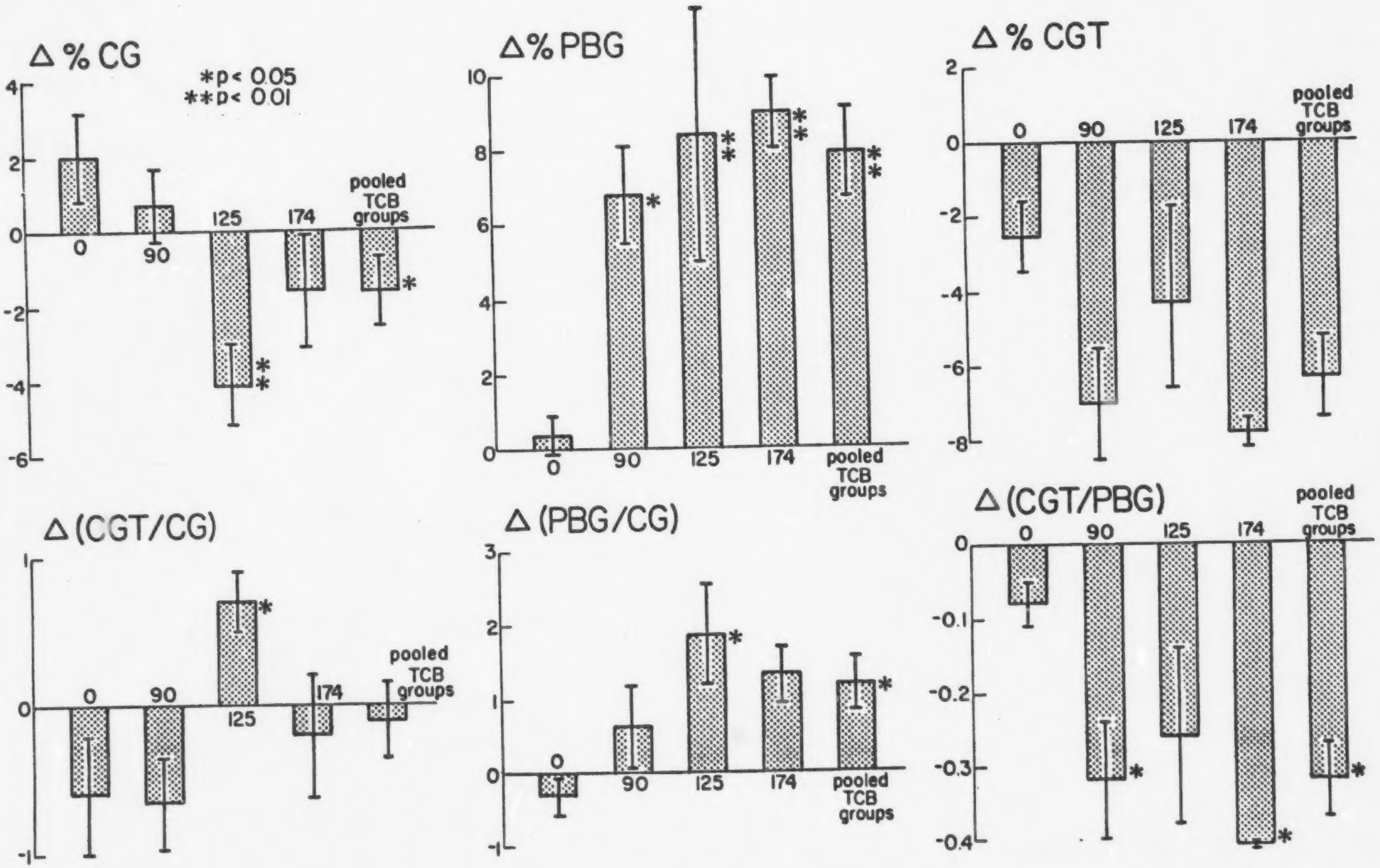
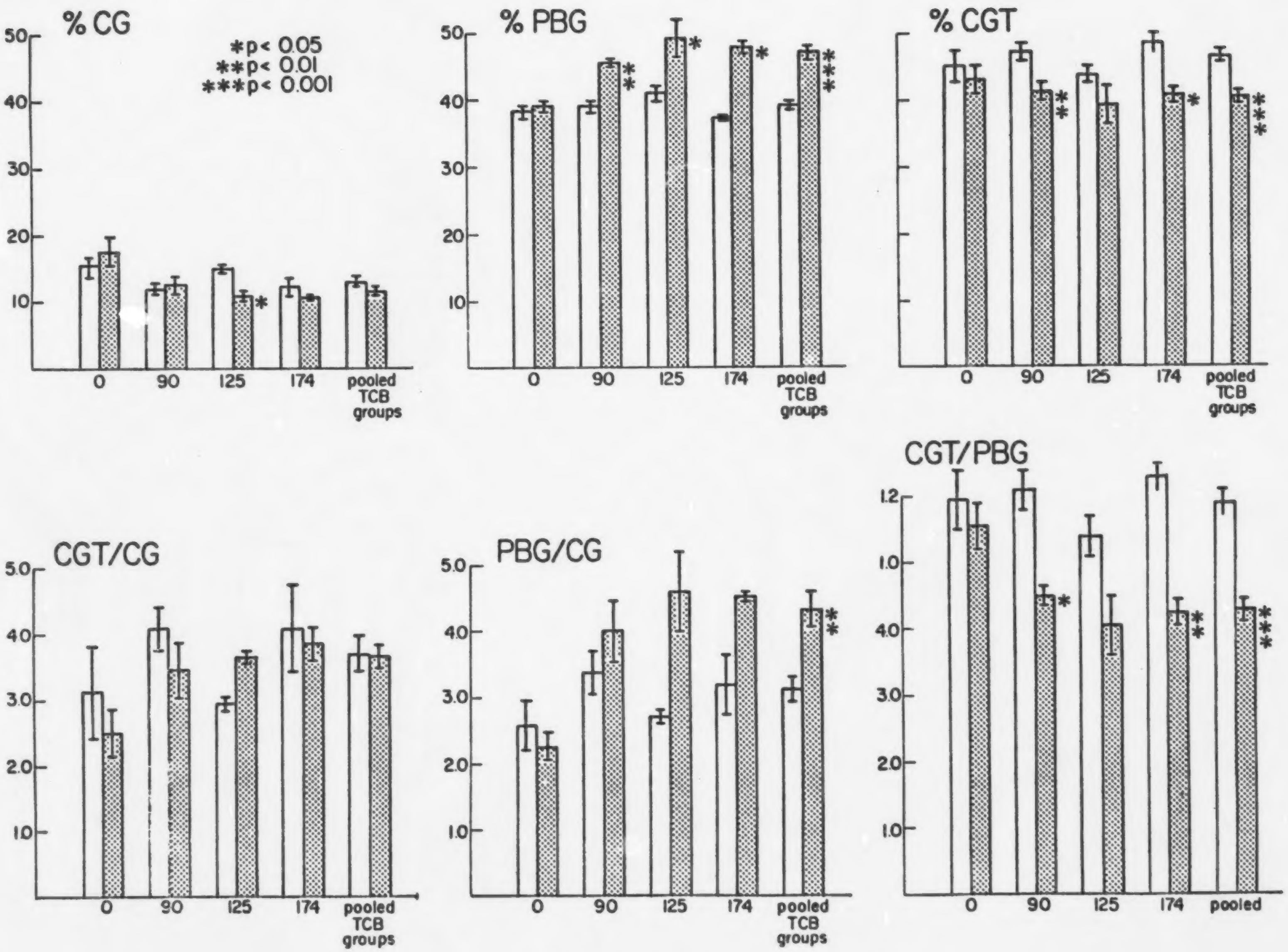


FIGURE 4

*EFFECT OF 30 DAYS TCB PRETREATMENT ON URINARY CHLORGUANIDE EXCRETION PARAMETERS*

*(post-treatment values compared to their own baselines by paired t test)*



# SECTION 5

## *IN VITRO* STUDIES ON TRICHLOROBENZENE

### I. *IN VITRO* ENZYME ASSAYS

### Preparation of Microsomal Suspensions

Liver samples were taken directly from liquid nitrogen and placed in a large beaker containing KCl-Tris buffer (0.15 M and .05 M , respectively, at ph 7.7). Samples and buffer were continually mixed inside large beakers which were placed in a large volume of water to help thaw the samples within 20 to 30 minutes. This process had to be performed rapidly and consistently since thawing time will affect mixed function oxidase (MFO) stability. All subsequent procedures (until incubation) were carried out at 0-5°C.

After samples were thawed, blotted dry and carefully weighed, 3 mls of ice cold KCl-Tris buffer (as above) were added per gram of liver. A "Tissuizer" (Tekmar Inc.) was used to blend buffer and liver and the resulting homogenate was spun down at 10,000 x g. for 20 minutes in a refrigerated Sorvall RC-2B centrifuge. This process was repeated on the supernatant whereupon 10 mls of the second supernatant were placed in ultracentrifuge tubes and spun at 100,000 x g. for 60 minutes in a refrigerated Beckman Spinco Model L2-65B preparative ultracentrifuge. Supernatant was discarded and the resulting pellet was resuspended in KCl-Tris buffer such that 1 ml was equivalent to 50 mg of whole liver tissue (20-fold dilution, w/v). The resulting microsomal suspension (on ice) was ready for *in vitro* MFO tests and had to be used within a few hours. In the carbon monoxide binding studies for the quantitation and characterization of MFO, phosphate buffer was substituted for KCl-Tris buffer. Protein concentrations of these microsomal suspensions were measured by the method of Lowry *et al.* ( 7 ) as modified by Miller *et al.* ( 8 ).

### In Vitro MFO Tests

MFO enzymatic activity (P-448 and P-450) was quantitated *in vitro* using four substrates which were incubated with microsomal preparations and essential cofactors. Aminopyrine and benzphetamine N-demethylation rates were assayed by measuring formation of formaldehyde after the Cochin and Axelrod method ( 9), as modified by Nash ( 10). Zoxazolamine hydroxylation activity was determined by monitoring disappearance of parent ( 11). The production of p-aminophenol was quantitated in the aniline hydroxylase reaction after the methods of Guarino *et al.* (12,13). Preliminary studies were conducted with monkey liver microsomes to insure linearity of the reaction rates of all four substrates over the incubation periods. Thus, kinetics were saturated and MFO was stable throughout the incubations. Reaction rates were reported either as nM of substrate converted per gm liver per minute or as nM per mg microsomal protein per minute. Absorptions were determined on a Perkin-Elmer 575 double beam spectrophotometer. This instrument was also used to quantitate MFO by carbon monoxide binding spectral analysis according to the method of Omura and Sato ( 14).

In Table 21 we report the results of the *in vitro* assays using four substrates, aminopyrine (AP), benzphetamine (BZ), zoxazolamine (ZX) and aniline (AL). The first two substrates are primarily N-demethylated while the last two are primarily hydroxylated. Inspection of the data in Table 21 shows that there were significant increases in activity against all four substrates. For AP the increases following 90 and 125 mg/kg TCB doses averaged 302 and 286%; for BZ, 331 and 302%; for ZX, 356 and 316%; and for AL, 364 and 294%. Although the averages for the monkeys on the 174 mg/kg dose were elevated, the data are complicated by overwhelming toxicity of the compound at this dose level requiring cessation of treatment. Thus, the possible inhibiting or potentiating effects of acute weight loss and reduced food intake cannot be evaluated from the data in this part of the experiment.

If one analyzes the data using a one-way analysis of variance (ANOVA) and eliminates high dose monkeys #39 and #343 from the analysis we obtain the data in Table 22. The "overall" F comparing TCB-treated groups to vehicle-treated controls has a P of 0.003 to 0.01. Evaluation of the contrasts indicated in the table show that the 90 mg/kg and 125 mg/kg doses are accompanied by significant increases in activity against all four substrates. The 174 mg/kg dose also produces increases in all the associated enzyme activities but the induction is not as great probably because the dose also produces both hepatic and general toxicity.

Similar results were obtained when the data are expressed on the basis of nmol/min/mg microsomal protein. The data are not quite as consistent (data not included for brevity) and one explanation of this may be the variability we observed with the Lowry microsomal protein procedure.

Data on the hepatic MFO concentrations present in each control and treated liver, based on CO binding studies, are shown in Table 23. The data are presented as nmols/gram of liver and nmols MFO/mg microsomal protein and the corresponding peak location of the MFO maxima in nm are also listed. One can see that the peak locations for the TCB-treated animals were indistinguishable from the controls but the activity in nmols was always increased (except for the toxic 174 mg/kg animals) and this was most prominent in the nmol/gr liver figures (Table 22).

Finally we have made some correlations (as scatter diagrams) between the various chloroguanide urinary excretion parameters such as %PBG, CGT, etc., and the various *in vitro* estimates of MFO activity using the data we obtained with aminopyrine, benzphetamine, zoxazolamine and aniline. Three of these correlations are presented in Figures 5 to 7. The figures were constructed using the values obtained for all treated animals on which we have *in vivo* CG excretion data and *in vitro* hepatic enzyme data. One observes the same general patterns with all three substrates presented. Without exception there is a positive correlation between urinary %PBG, increase in PBG excretion ($\Delta$ PBG) and the $\Delta$ PBG-$\Delta$ CGT values.

A negative correlation (as one would have anticipated) was observed when the enzyme activity data were plotted against %CGT, CGT/PBG, $\Delta$ CGT, $\Delta$ CGT/PBG) and $\Delta$ PBG x $\Delta$ CGT. Although we attach no great weight to the

TABLE 21. RESULTS OF IN VITRO HEPATIC MFO TESTS IN RHESUS MONKEYS TREATED WITH TCB

(NANOMOLE/MINUTE/GRAM LIVER)

| TCB Dose mg/kg/day | Monkey No. | Aminopyrine Demethylase | Benzphetamine Demethylase | Zoxazolamine Hydroxylase | Aniline Hydroxylase |
|---|---|---|---|---|---|
| 0 | 573 | 76.6 | 155 | 32.4 | 14.2 |
| | 483 | 53.2 | 120 | 6.90 | 11.4 |
| | 366 | 55.5 | 131 | 19.8 | 11.7 |
| | 495 | 54.4 | 123 | 19.0 | 12.0 |
| 90 | 634 | 199 | 462 | 67.2 | 31.8 |
| | 505 | 165 | 376 | 60.3 | 35.0 |
| | 110 | 192 | 535 | 96.4 | 61.8 |
| | 500 | 168 | 383 | 53.4 | 50.5 |
| 125 | C18 | 192 | 435 | 65.2 | 35.0 |
| | C14 | 125 | 262 | 49.4 | 19.9 |
| | C12 | 173 | 393 | 55.4 | 31.4 |
| | 253 | 195 | 508 | 76.1 | 58.4 |
| 174 | 287 | 142 | 375 | 74.1 | 42.4 |
| | 39* | 73.8 | 158 | 17.8 | 12.1 |
| | 343 | 76.6 | 180 | 19.3 | - |
| | 276 | 64.4 | 256 | 29.2 | 40.9 |

*This monkey had been withdrawn from treatment for over 1 month prior to sacrifice.

TABLE 22. SIGNIFICANCE LEVELS (P) COMPARING IN VITRO MFO ACTIVITY AMONG CONTROL AND TCB TREATED GROUPS*

(NANOMOLES/MINUTE/GRAM LIVER)

| Comparison | Aminopyrine Demethylase | Benzphetamine Demethylase | Zoxazolamine Hydroxylase | Aniline Hydroxylase |
|---|---|---|---|---|
| "Overall" F | p = 0.0003 | 0.0008 | 0.0089 | 0.0136 |
| ........ Contrasts ........ | | | | |
| Control vs. Pooled TCB Treated Groups | 0.0002 | 0.0002 | 0.0022 | 0.0023 |
| Dose-Response | 0.0079 | 0.0872 | 0.2560 | 0.7647 |
| Curvilinearity | 0.1355 | 0.6645 | 0.9274 | 0.3857 |
| Controls vs. 90's | 0.0001 | 0.0002 | 0.0018 | 0.0029 |
| Controls vs. 125's | 0.0002 | 0.0005 | 0.0054 | 0.0165 |
| Controls vs. 174's | 0.0955 | 0.0184 | 0.0514 | 0.0161 |

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*Significance levels were derived by a one-way ANOVA program capable of contrast evaluation.

TABLE 23. *IN VITRO* HEPATIC ENZYME TESTS IN RHESUS MONKEYS TREATED WITH TCB

(NANOMOLE/MINUTE/MG MICROSOMAL PROTEIN)

| TCB Dose mg/kg/day | Monkey No. | Aminopyrine Demethylase | Benzphetamine Demethylase | Zoxazolamine Hydroxylase | Aniline Hydroxylase |
|---|---|---|---|---|---|
| 0 | 573 | 2.18 | 4.42 | 0.92 | 0.36 |
| | 483 | 1.87 | 4.21 | 0.24 | 0.40 |
| | 366 | 1.61 | 3.80 | 0.57 | 0.34 |
| | 495 | 1.39 | 3.14 | 0.48 | 0.26 |
| 90 | 634 | 4.02 | 9.33 | 1.36 | 0.64 |
| | 505 | 3.60 | 8.18 | 1.31 | 0.76 |
| | 110 | 4.81 | 13.4 | 2.42 | 1.56 |
| | 500 | 4.17 | 9.50 | 1.32 | 1.08 |
| 125 | C18 | 3.77 | 8.53 | 1.28 | 0.69 |
| | C14 | 2.92 | 6.14 | 1.16 | 0.49 |
| | C12 | 3.75 | 8.54 | 1.20 | 0.68 |
| | 253 | 4.69 | 12.2 | 1.82 | 1.16 |
| 174 | 287 | 3.18 | 8.40 | 1.66 | 0.87 |
| | 39* | 2.02 | 4.32 | 0.49 | 0.33 |
| | 343 | 2.89 | 6.79 | 0.73 | - |
| | 276 | 1.75 | 6.99 | 0.80 | 0.77 |

* This monkey had been withdrawn from treatment for over 1 month prior to sacrifice.

TABLE 24. SIGNIFICANCE LEVELS (P) WHICH COMPARE IN VITRO MFO ACTIVITY OF TCB TREATED MONKEYS TO CONTROLS

(NANOMOLES/MINUTE/MG MICROSOMAL PROTEIN)

| Comparison | Aminopyrine Demethylase | Benzphetamine Demethylase | Zoxazolamine Hydroxylase | Aniline Hydroxylase |
|---|---|---|---|---|
| "Overall" F | p = 0.0009 | 0.0053 | 0.0295 | 0.0390 |
| ............ Contrasts ............ | | | | |
| Control vs. Pooled TCB Treated Groups | 0.0009 | 0.0014 | 0.0075 | 0.0105 |
| Dose-Response | 0.0095 | 0.1742 | 0.3328 | 0.4464 |
| Curvilinearity | 0.2633 | 0.9714 | 0.8581 | 0.4025 |
| Controls vs. 90's | 0.0002 | 0.0010 | 0.0056 | 0.0065 |
| Controls vs. 125's | 0.0008 | 0.0042 | 0.0215 | 0.0597 |
| Controls vs. 174's | 0.2106 | 0.0437 | 0.0934 | 0.0727 |

*Significance levels were derived by a one-way ANOVA program capable of contrast evaluation.

FIGURE 5. AMINOPYRINE N-DEMETHYLASE ACTIVITY (AS nM/GM LIVER/MIN) CORRELATED WITH EIGHT CHLORGUANIDE URINARY EXCRETION PARAMETERS

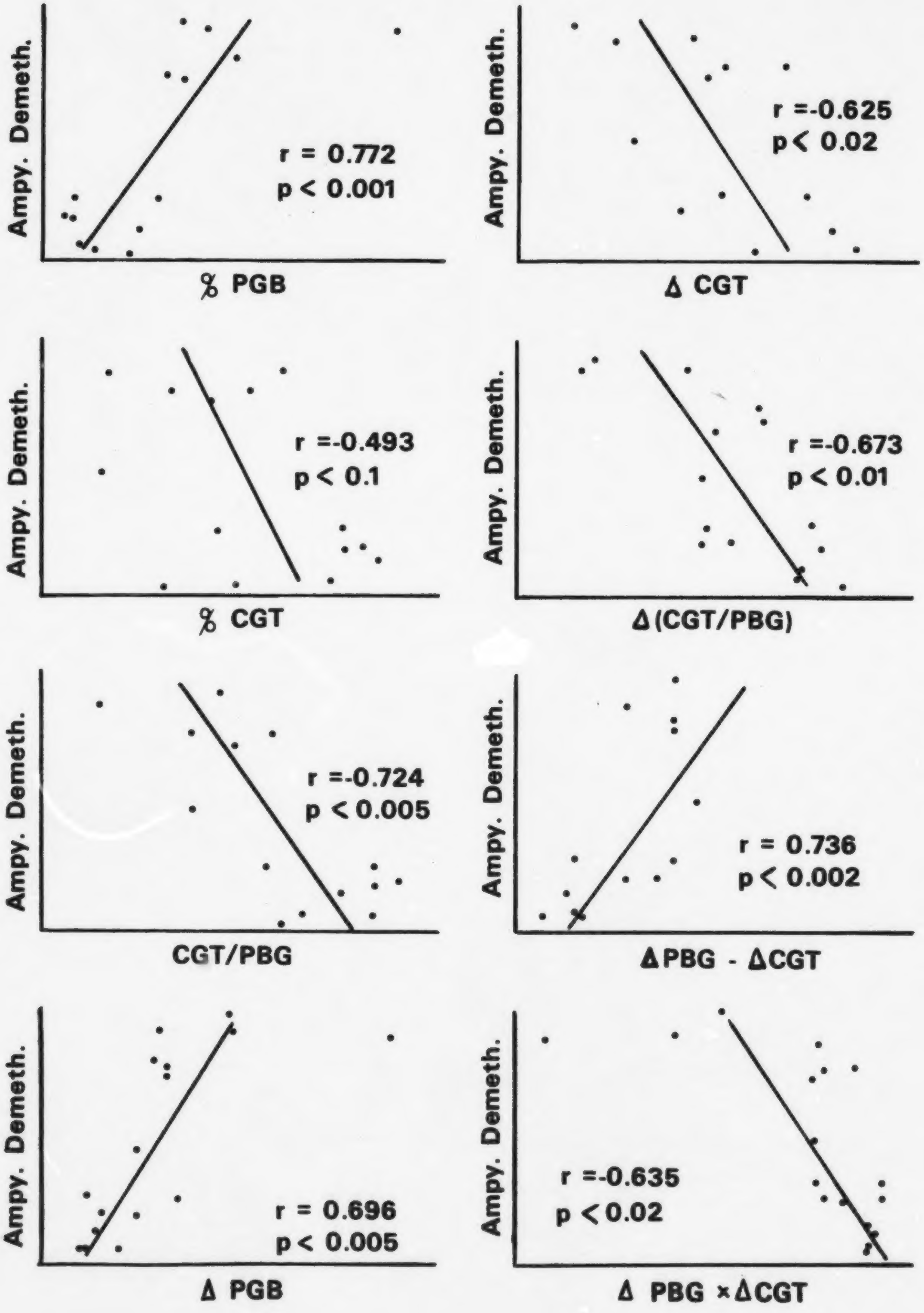


FIGURE 6. BENZPHETAMINE N-DEMETHYLASE ACTIVITY (AS NM/GM LIVER/MIN) CORRELATED WITH EIGHT CHLORGUANIDE URINARY EXCRETION PARAMETERS

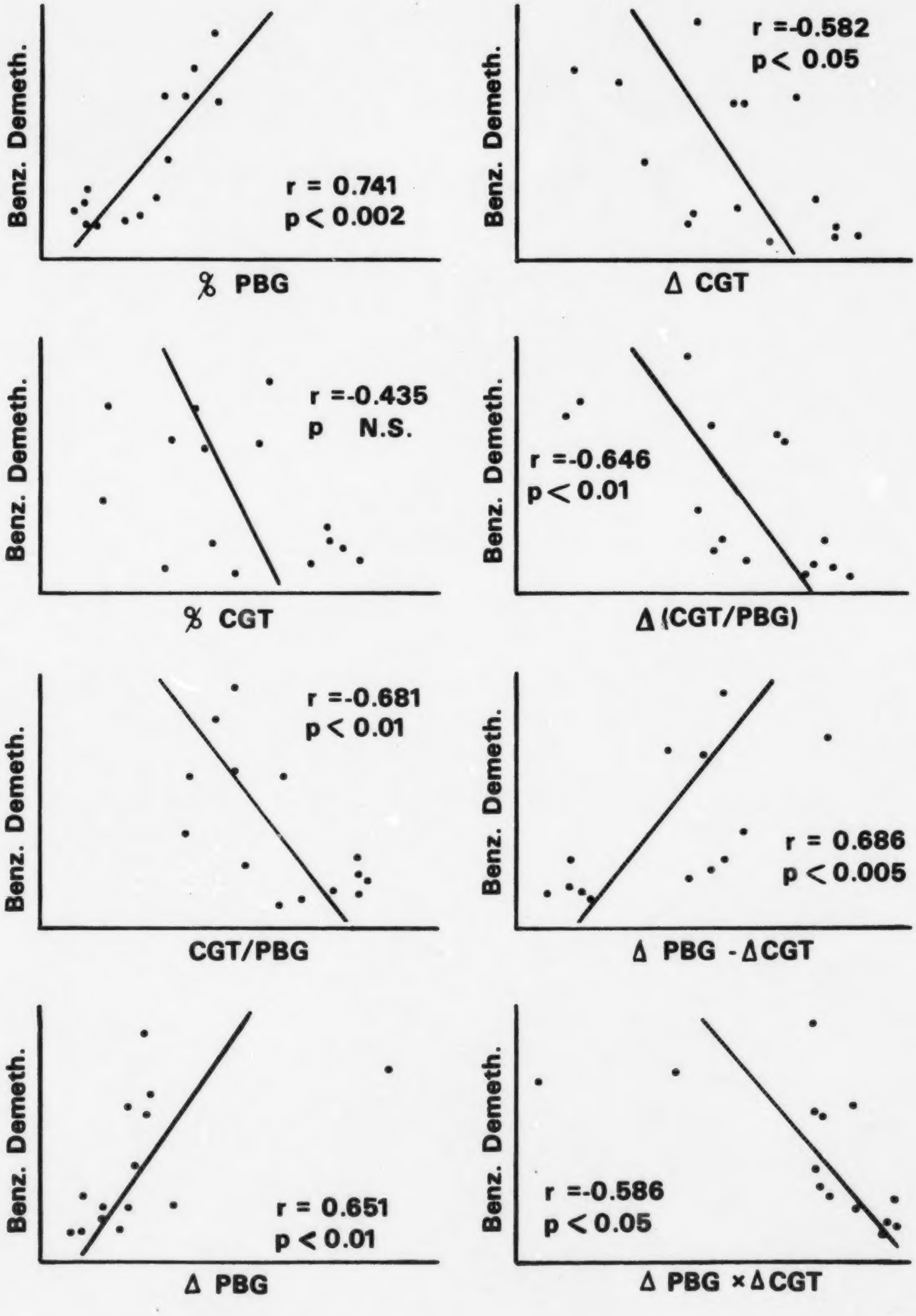
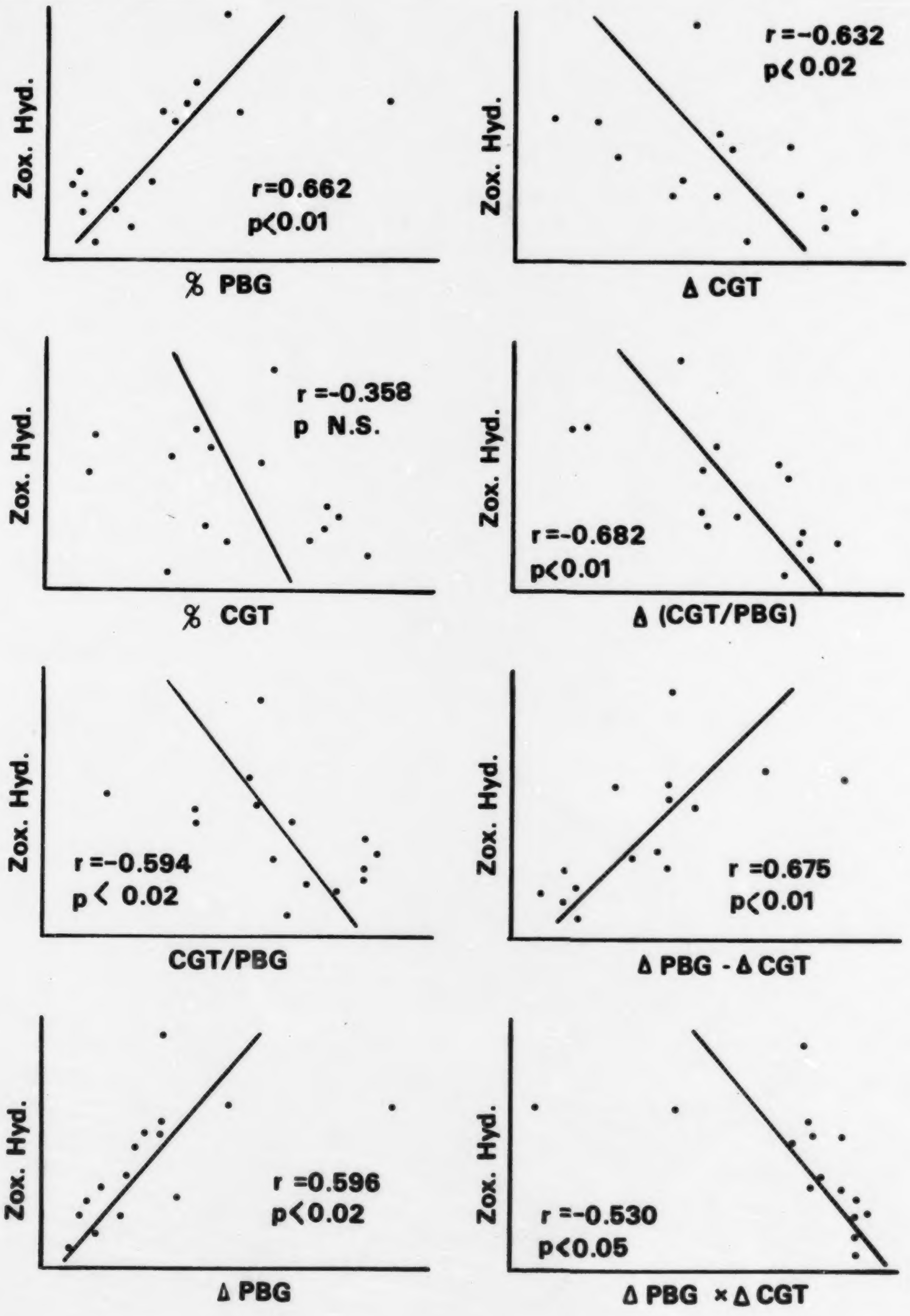


FIGURE 7. ZOXAZOLAMINE HYDROXYLASE ACTIVITY (AS nM/GM LIVER/MIN) CORRELATED WITH EIGHT CHLORGUANIDE URINARY EXCRETION PARAMETERS



## TABLE 25. MFO CONCENTRATIONS AND PEAK LOCATIONS FROM CONTROL AND TCB TREATED MONKEYS

| Treatment | Monkey No. | nM MFO / g liver | nM MFO / mg mic prot | Peak Location (nm) |
|---|---|---|---|---|
| Emulphor Vehicle | 573 | 25.5 | 0.724 | 451.0 - 452.0 |
| | 483 | 18.0 | 0.632 | 451.5 - 452.5 |
| | 366 | 29.9 | 0.866 | 451.0 - 452.0 |
| | 495 | 24.2 | 0.617 | 451.0 - 452.0 |
| 90 mg/kg TCB | 634 | 56.3 | 1.11 | 451.0 - 452.5 |
| | 505 | 60.0 | 1.30 | 451.0 - 452.0 |
| | 110 | 61.0 | 1.54 | 451.0 - 452.0 |
| | 500 | 40.2 | 0.998 | 451.0 - 452.0 |
| 125 mg/kg TCB | C18 | 54.9 | 1.08 | 451.0 - 452.5 |
| | C14 | 48.8 | 1.06 | 451.0 - 452.0 |
| | C12 | 48.8 | 1.06 | 451.0 - 452.0 |
| | 253* | - | - | - |
| 174 mg/kg TCB | 287* | - | - | - |
| | 39* | 24.2 | 0.662 | 451.0 - 452.5 |
| | 343* | 13.8 | 0.360 | 451.0 - 452.0 |
| | 276 | 34.7 | 0.946 | 451.5 - 452.5 |

*Monkeys not used in statistical evaluation.

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## TABLE 26. STATISTICS COMPARING MFO CONCENTRATIONS (AS NM/G LIVER) AMONG CONTROL AND TCB TREATED GROUPS*

| Comparison | F | df's | p |
|---|---|---|---|
| "Overall" F | 13.7 | 2,8 | 0.0016 |
| ................ Contrasts ................ | | | |
| Control vs. Pooled TCB Treated Groups | 30.2 | 1,8 | 0.0006 |
| Dose-Response | 6.12 | 1,8 | 0.0380 |
| Curvilinearity | 0.11 | 1,8 | 0.7492 |
| Controls vs. 90's | 35.7 | 1,8 | 0.0003 |
| Controls vs. 125's | 21.6 | 1,8 | 0.0017 |
| Controls vs. 174's | 1.69 | 1,8 | 0.2291 |

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[*] Statistics were derived by a one-way ANOVA program capable of contrast evaluation.

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indicated "f" and "p" values which were calculated using standard procedures, it is intriguing to recognize that these correlations are all in the direction we would have anticipated based on the hypothesis that increase in hepatic MFO activity of the P-450 type should increase PBG excretion and reduce CGT excretion and possibly CG as well.

These data have also been evaluated nonparametrically using the Wilcoxon Signed Rank and the Wilcoxon Rank Sum Tests ( 15). Although the charts showing these nonparametric analyses of the data are not shown (for brevity) they indicated a high degree of significance between the treatment with TCB and changes in the urinary excretion patterns of CG metabolites. These analyses can be made available if desired. These results in conjunction with the ANOVA analyses shown previously support the conclusion that daily administration of TCB to monkeys in doses of 90 to 174 mg/kg for 2 or 3 months causes a definite induction of hepatic MFO activity and this increased activity can be rather accurately predicted using the new *in vivo* CG excretion procedure described in this paper.

## II. EXTRACTION OF TCB METABOLITES FROM RAT URINE

### Preliminary Data

A pooled 24-hour treatment urine sample containing 1 N HCl was refluxed for one hour. This hydrolyzed urine pool was then buffered to pH 2 with 0.4 M phosphate and 1 ml aliquots were extracted with 10 ml of various organic solvents listed in Table 27. A duplicate set of aliquots was run which, in addition contained 1 gram of $(NH_4)_2SO_4$.

As shown in Table 27 addition of salt increased the extraction efficiency of most solvents. The solvents ranked by decreasing extraction efficiency as follows: n-amyl alcohol > 2 ethyl hexanol > EDC + EtHex > EDC = ethyl ether > $CHCl_3$ > benzene = Toluene > $CCl_4$ > petroleum ether > cyclohexane. At present the best solvents appear to be the higher alcohols. We assume the urine contained TCB metabolites such as phenols and dihydrodiols and catechols. These were probably present in the original urine samples as conjugates.

We also carried out chromatographic studies of hydrolyzed urine and urine extracts using both TLC and paper chromatography. The anticipated TCB metabolites were: 2,3,5-trichlorophenol (2,3,5-TCP), 2,4,5-trichlorophenol (2,4,5-TCP), 2,3,6-trichlorophenol (2,3,6-TCP), and 2,4,5-trichlorothiophenol (2,4,5-TCTP). They could be visualized using diazotized p-nitroaniline and diazotized sulfanilic acid and gave the colors shown in Table 28.

## TABLE 27. EXTRACTION OF $^{14}C$-LABELED METABOLITES OF TRICHLOROBENZENE IN HYDROLYZED RAT URINE BY VARIOUS ORGANIC SOLVENTS

EFFECT OF ADDED $(NH_4)_2SO_4$

| Solvent (10 ml) | Pooled Urine | % $^{14}C$ Extracted | |
|---|---|---|---|
| | | No Salt | $(NH_4)_2SO_4$ |
| n-amyl alcohol | 1 ml | 87.7 | 90.9 |
| 2-ethylhexanol | " | 77.8 | 82.6 |
| EDC + 2 ethylhexanol (8:2) | " | 64.5 | 73.8 |
| ethylene dichloride (EDC) | " | 40.2 | 53.9 |
| ethyl ether | " | 45.7 | 49.9 |
| $CHCl_3$ | " | 35.1 | 39.6 |
| benzene | " | 26.5 | 28.8 |
| toluene | " | 23.1 | 27.8 |
| $CCl_4$ | " | 15.6 | 19.5 |
| petroleum ether | " | 8.8 | 10.2 |
| cyclohexane | " | 8.3 | 10.0 |

TABLE 28. CHROMOGENIC REACTIONS

| Standard | diazotized p-nitroaniline | diazotized sulfanilic acid |
|---|---|---|
| 2,3,5-TCP | Orange | Orange |
| 2,3,5-TCP | Violet | Violet |
| 2,3,6-TCP | Magenta | Yellow |
| 2,4,5-TCTP | Yellow | Yellow |

The solvent systems employed were (A) benzene - acetic acid - water (1:1:2; v/v/v) and (B) N-butanol - ethanol - 1.5 N ammonium acetate (40:11:19; v/v/v). The $R_f$ values for the postulated metabolites in the two solvent systems using either cellulose or silica gel are shown in Table 29.

TABLE 29. $R_f$ VALUES

| | Solvent System A | | Solvent System B | |
|---|---|---|---|---|
| Standard | Cellulose | Silica Gel | Cellulose | Silica Gel |
| 2,3,5-TCP | 0.84 | 0.29 | 0.89 | 0.63 |
| 2,4,5-TCP | 0.92 | 0.29 | 0.91 | 0.66 |
| 2,3,6-TCP | 0.89 | 0.23 | 0.80 | 0.61 |
| 2,4,5-TCTP | 0.71 | 0.69 | 0.83 | 0.70 |

An interfering red background in solvent system B could be removed by spraying with 1 N $H_2SO_4$ several minutes after $Na_2CO_3$ spraying.

## Conclusions From TLC and Paper Chromatography

Results from both the TLC and paper chromatography studies (data not shown) indicated the presence of at least two metabolites (or classes of metabolites) which chromatograph differently from the available standards. Solvent extracts from urine chromatographed on cellulose plates in solvent system A (nonpolar) showed considerable radioactivity at the origin. This activity could not be glucuronide or sulfate conjugates, since this urine had been acid hydrolyzed for one hour. Therefore, it appears that metabolite(s) which remain at the origin with the nonpolar TLC system must represent unconjugated metabolites which are more polar than any of the standards.

## Gas-Liquid Chromatography

Utilizing a Varian Aerograph Series 2100 gas-liquid chromatograph, 2,3,5-; 2,4,5-; and 2,3,6-TCP and 2,4,5-TCTP standards were separated with a 6 ft. SE 30; 100-120 mesh column (inside diameter 2 mm). Standards were detected by electron capture ($^{63}$Ni source) in quantities as small as 1 nanogram. Inlet temperature was initially 300°C and column temperature was 100°C. Flow rate was 40 ml $N_2$/min. Standards exhibited retention times shown in Table 30.

TABLE 30. GC RETENTION TIMES

| Standard | $R_T$ (min.) | corresponding peaks from ether extract |
|---|---|---|
| 2,3,5-TCP | 4.09 | yes |
| 2,4,5-TCP | 4.88 | yes |
| 2,3,6-TCP | 5.20 | no |
| 2,4,5-TCTP | 8.03 | yes |

Concentrated ether extracts (100x) of unhydrolyzed urine produced peaks corresponding to 2,3,5- and 2,4,5-TCP. No peaks corresponded to the 2,3,6-TCP standard. A sharp peak with an identical retention time as the 2,4,5-TCTP was also observed; however, this was unexpected from unhydrolyzed urine, since thiophenolic metabolites are usually considered to be degradation products from mercapturates after hydrolysis under strongly acidic or basic reflux conditions. It was surmised that high inlet port temperature could be degrading mercapturate which had been extracted into ether (these conjugates are slowly extractable into ether). When the inlet port temperature was dropped from 300°C to 130°C, the presumptive thiophenol peak disappeared while standard thiophenol peak was still visible at the same $R_T$. The inlet port temperature of 130°C still left the two peaks corresponding to the above-mentioned phenols intact and at their respective $R_T$'s. It is, therefore, tentatively concluded that at an inlet port temperature of 300°C, the peak corresponding to 2,4,5-trichlorothiophenol is derived from the thermally degraded mercapturate.

# III. PRELIMINARY CHARACTERIZATION OF METABOLITES OF TCB IN MONKEY PLASMA

## Extraction of Monkey Plasma

One ml each of 0,1,2, and 4 hour plasma samples from a monkey receiving

10 mg/kg TCB was added slowly to 4 ml of 10% perchloric acid (PCA) and mixed rapidly on a vortex to insure precipitation of all proteins. The PCA-precipitated plasma was extracted with 5 ml hexane 3 times. Each hexane extract was analyzed for total radioactivity and the percent $^{14}$C extracted was calculated. Following the third hexane extract, the plasma/PCA residue was extracted 3 times with 5 ml portions of ethyl ether.

After drying over anhydrous $Na_2SO_4$, the hexane extracts were analyzed by GC-EC ($^{63}$Ni-detector) on a 6' x 2 mm i.d. column packed with 100-120 mesh Veriport® coated with 3% SE-30; carrier gas, $N_2$ at 30 ml/min; column temperature 100°C; injection port, 300°C; detector over, 290°C; sensitivity 2 x $10^{-10}$ amps/mv.

## Results

Recovery based on radioactivity analysis of the hexane and ether extracts is shown in Table 31.

TABLE 31. RECOVERY CF RADIOACTIVITY FROM MONKEY PLASMA IN PERCENT

| Time in hr | Hexane Soluble | Ether Soluble | Total Extracted |
|---|---|---|---|
| 1 | 52.5 | 48 | 100.5 |
| 2 | 41.5 | 50.5 | 92.0 |
| 4 | 30.5 | 63.0 | 93.5 |

GC analysis of the first hexane extracts revealed only one peak not present in parallel extracts of pretreatment plasma. This peak had the same retention time as 1,2,4-trichlorobenzene (TCB). Attempts to characterize the ether extractable material by GLC were not successful and further research in this area was not carried out.

## IV. INHIBITION AND POTENTIATION OF THE HEPATOTOXICITY OF 1,2,4-TRICHLOROBENZENE IN RATS

The following study was undertaken to compare the effects of two classical MFO inducers, phenobarbital (PB) and 3-methylcholanthrene (3-MC) on the acute toxicity of TCB.

## Materials and Methods

Twenty male Charles River rats were divided into groups of four and pretreated with phenobarbital (PB), Aroclor® 1254 (PCB) or 3-methylcholanthrene (3-MC) as shown in Table 32 below. In each group, two animals received a single dose of 800 and 2 were given 1000 mg/kg of TCB orally (in corn oil).

TABLE 32. TREATMENT PROTOCOL

| Group | Pretreatment | Pretreatment dose per day | No. Days Pretreatment |
|---|---|---|---|
| 1 | $H_2O$ | 2 ml/kg | 4 |
| 2 | PB in $H_2O$ | 75 mg/kg | 4 |
| 3 | Corn Oil | 2 ml/kg | 5 |
| 4 | PCB in Corn Oil | 50 mg/kg | 5 |
| 5 | 3-MC in Corn Oil | 20 mg/kg | 2 |

TCB was administered 24 hours after the last pretreatment and the rats were sacrificed six days later for histological examination. Animals were weighed daily throughout the entire experiment. The two high dose (1000 mg/kg) PCB-pretreated rats died within 24 hours after TCB administration. One low dose PCB rat and one high dose 3-MC-induced rat died four and three days after TCB administration, respectively. All other rats survived the period of observation. PCB and 3-MC pretreated rats lost more weight than either $H_2O$ or corn oil pretreated control rats. Phenobarbital (PB) pretreated rats unexpectedly lost less weight than their respective controls. All rats which survived had maximal weight loss at days three or four and then began to gain. Histologic examination indicated that the most severe liver damage occurred in those groups which lost the most weight. PB-treated rats showed less liver damage than the $H_2O$-treated controls.

## Discussion

The observation that PB pretreatment protected against TCB hepatotoxicity is contrary to the findings of Reid et al. ( 16 ) who found that PB-induction potentiated the toxicity of lower halogenated compounds fluoro-, chloro-, bromo-, indo-, 1,2,dichloro-, and 1,3-dichlorobenzene. Potentiation of TCB toxicity by 3-MC pretreatment in our study is also directly opposite to the observations of Conney, Miller and Miller ( 17) on chlorobenzene and Jollow, Zampaglione and Gillette ( 18) for bromobenzene. These investigators found that 3-MC induction had a protective effect on the hepatotoxicity of chloro- or bromobenzene.

## Conclusion

Phenobarbital decreased and 3-methylcholanthrene increased the toxicity of single oral doses of TCB. Observations on the histology of the livers of

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the various groups suggested that the basis for the inhibition (PB) or potentiation (3-MC) was probably related to the changes in hepatotoxicity.

## V. CYTOTOXICITY AND MUTAGENICITY OF TCB METABOLITES

With the assistance of Mr. Steve Glasser in Dr. John C. Loper's laboratory we have examined the cytotoxicity and mutagenicity of some of the known or potential metabolites of 1,2,4-trichlorobenzene (TCB) using two of the sensitive Ames Salmonella typhimurium tester strains TA98 and TA100 ( 20 ). A dose-response curve was carried out for each compound both with and without added liver microsomes (S9) derived from rats treated optimally with PCB's.

The data obtained from duplicate plates are summarized in Table 33. It is obvious that although the compounds are generally toxic in the highest concentrations used no increase in mutation rate was obtained with non-toxic concentrations. Also, TCB, the parent compound, had no mutagenic activity with or without added liver microsomes based on data obtained previously in Dr. Loper's laboratory.

The data in Table 34 are presented only to show that the tester strains are sensitive to the three known mutagens, methylmethane sulfonate, nitroquinoline N-oxide and 2-aminoanthracene.

We must conclude that TCB or its known or potential phenolic metabolites are inactive as mutagens. Furthermore, combination of TCB and active liver microsomes failed to produce any increase in mutation frequency in this sensitive test system.

TABLE 33. TOXICITY (T) OR ACTIVITY OF TCB METABOLITES

| | µg/plate in DMSO | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 100 | | 10 | | 1 | | .01 | |
| | T98 | T100 | T98 | T100 | T98 | T100 | T98 | T100 |
| 2,3,4 Trichlorophenol | T | T | - | - | - | - | - | - |
| 2,3,5 Trichlorophenol | T | T | - | - | - | - | - | - |
| 2,3,6 Trichlorophenol | T | T | T | - | - | - | - | - |
| 2,4,5 Trichlorophenol | T | T | - | - | - | - | - | - |
| 2,4,6 Trichlorophenol | T | T | - | - | - | - | - | - |
| 3,4,5 Trichlorophenol | T | T | - | - | - | - | - | - |
| 2,4,5 Trichlorothiophenol | T | - | - | - | - | - | - | - |
| 2,4 Dichlorophenol | ±T | - | ±T | - | ±T | - | - | - |
| 2,5 Dichlorophenol | T | T | - | - | - | - | - | - |
| 2,5 Dichlorohydroquinone | T | T | T | T | T | T | - | T |

T = toxic

- = no increase in mutation rate over the spontaneous control rate. Presence or absence of S9 fraction had no significant effect on the results and therefore is not indicated.

## TABLE 34. CONTROL MUTAGENICITY TEST DATA USING AMES TESTER STRAINS TA98 AND TA100

| Chemical Added | TA98 |  | TA100 |  |
|---|---|---|---|---|
|  | -S9* | +S9 | -S9 | +S9 |
| Spontaneous mutation rate | 23 | 32 | 117 | 115 |
| Methyl methane sulfonate |  |  | 1644 |  |
| Nitroquinoline N-oxide | 156 |  |  |  |
| 2-aminoanthracene |  | 3079 |  | 2114 |

*Liver microsomes (S-9) from rat liver induced with PCB.

# SECTION 6

## METABOLISM OF BROMODICHLOROMETHANE IN RATS AND RHESUS MONKEYS

### I. INTRODUCTION

Bromodichloromethane (BDC) is one of the halo-organics found commonly in potable water following chlorination ( 19). It is an analogue of chloroform and very little data are available concerning its metabolic fate in laboratory animals. This report contains data on the pharmacokinetics and tissue distribution of BDC in rats and rhesus monkeys.

### II. PRELIMINARY PHARMACOKINETIC STUDIES ON BROMODICHLOROMETHANE (BDC) IN RATS

Our first study of BDC consisted of examining the tissue distribution and excretion of the compound following single intravenous or oral doses in four male rats. For this pilot study we chose sacrifice times of three and six hours, time periods which we hoped would bracket the period of peak tissue levels.

#### Materials and Methods

Four Charles River male albino rats weighing 480-587 g were used. Two rats were dosed orally and sacrificed at three and six hours; similarly, two rats were dosed intravenously and sacrificed at three and six hours.

The treatment solution prepared for the intravenous studies contained 200 µl $^{14}$C-BDC (0.5 mCi/ml) and 50 µl cold BDC dissolved in 10 ml Emulphor®: ethanol (1:1,v/v) and diluted to 50 ml with distilled water. The resulting solution contained 9.90 mg of BDC and 2.0 µCi of labeled compound per ml. The intravenous dose was given via the femoral vein exposed under light ether anesthesia. These rats were given 10 mg BDC per kg.

To treat rats orally, a 1:5 dilution of the treatment solution described above was prepared. The solution contained 2.0 mg/ml and 0.4 µCi/ml of bromodichloromethane. The oral dose was 20 mg/kg.

Animals were housed in stainless steel metabolism cages. Urines were collected in iced containers until the animals were killed; no feces were excreted.

#### Results

One notes from the data in Table 35 that when BDC was given to rats intravenously it tended to accumulate in fat. Lower but significant concentrations were observed in liver, blood, plasma, kidneys, and lungs. There are small but important amounts in the GI tract, suggesting that the compound is excreted in the bile. When one compares the three and six hour rats one observes that the peak concentrations usually occur at three hours but in blood and plasma, liver, kidney, muscle, and GI tract the opposite holds true. In terms of percent dose, the fat accounts for more than 50% of the dose recovered in the tissues. Very little $^{14}$C is excreted in the urine or in the feces, even if one includes the contents of the GI tract.

When BDC was administered to rats orally the data in Table 36 indicate a quite different distribution pattern. The compound was slow to leave the stomach and concentrations in fat were high but lower than when the compound was administered intravenously. The $^{14}$C content of the liver was high (probably because of first pass effects) but when one compares oral with intravenous administration one sees that the tissues accounted for much less of the dose when the compound is given orally.

## III. PHARMACOKINETICS OF BDC IN RHESUS MONKEYS

Two female rhesus monkeys were used in a "cross-over" study of blood levels, excretion, and tissue distribution of BDC. Each monkey received single oral and intravenous doses of 10 mg/kg. No. 3 received the intravenous dose initially and an oral dose 9 days later. Finally after 82 days another oral dose was administered and 24 hours later the monkey was necropsied for tissue distribution of BDC-derived $^{14}$C. No. 13 received the oral dose initially; single intravenous doses were injected 9 and 82 days later. After 24 hours the monkey was necropsied and tissue levels of $^{14}$C were determined.

### Materials and Methods

The intravenous treatment solution was prepared by adding 200 µl of $^{14}$C-BDC (0.5 mCi/ml) and 50 µl BDC (cold) to 10 ml Emulphor®-ethanol (1:1, v/v) and diluting to 50 ml with distilled water. The resulting solution contained 9.9 mg/ml and 1.34 µCi/ml of BDC as determined by replicate assays.

This solution was diluted 1:5 with Emulphor®:ethanol:water (1:1:8, v/v/v) to prepare the oral treatment solution which contained 1.98 mg/ml and 0.147 µCi/ml of BDC as determined by replicate assays.

For the two initial treatments both monkeys were tranquilized with Ketamine HCl (5 mg/kg). For the terminal treatments both monkeys were simply restrained by the animal technician during the treatment procedures.

Monkey No. 3 weighing 4.95 kg received 5 ml of the intravenous treatment solution and monkey No. 13 weighing 4.6 kg received 4.6 ml of the intravenous treatment solution. For the final intravenous treatment No. 13 R weighed 4.93 kg and received 5.17 ml of the intravenous treatment solution. The first intravenous doses were administered via the cephalic vein. The final intravenous dose was administered to No. 13 R via the saphenous vein of the left leg. No unfavorable response was observed in either monkey.

The oral dose for each monkey was 25.0 ml of the oral treatment solution administered intragastrically using a rubber catheter. The catheter

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## TABLE 35. TISSUE DISTRIBUTION OF BROMODICHLOROMETHANE (AS $^{14}C$) IN MALE RATS

SINGLE INTRAVENOUS DOSE 10 MG/KG

| | Percent Dose | | µg/g | |
|---|---|---|---|---|
| Tissue | 3 hours | 6 hours | 3 hours | 6 hours |
| Blood | 0.89 | 1.00 | 1.06 | 1.12 |
| Plasma | - | - | 1.3 | 1.8 |
| Liver | 0.96 | 1.75 | 3.7 | 6.1 |
| Spleen | 0.01 | 0.01 | 0.78 | 0.62 |
| Kidney | 0.14 | 0.17 | 2.1 | 3.0 |
| Lungs | 0.06 | 0.04 | 2.1 | 1.4 |
| Heart | 0.03 | 0.03 | 1.09 | 0.94 |
| Testes | 0.05 | 0.03 | 0.69 | 0.44 |
| Brain | 0.04 | 0.01 | 0.93 | 0.39 |
| Fat* | 18.0 | 12.8 | 24.4 | 16.2 |
| Muscle** | 2.9 | 3.5 | 0.78 | 0.89 |
| Skin+ | 2.5 | 1.10 | 1.6 | 0.66 |
| Total Tissue | 25.5 | 20.5 | | |
| Stomach | 0.12 | 0.08 | 1.5 | 1.3 |
| Small Intestine | 0.50 | 1.00 | 2.4 | 5.8 |
| Cecum | 0.48 | 0.61 | 3.5 | 4.1 |
| Large Intestine | 0.23 | 0.30 | 4.1 | 4.2 |
| GI Tract & Contents | 1.3 | 2.0 | | |
| Recovery in Excreta | | | | |
| Urine | 0.14 | 0.32 | 3.2 | 1.7 |
| Feces | NS | NS | NS | NS |

NS = No sample.
*Fat = quantitated as 8% body wt.
**Muscle = quantitated as 40% body wt.
+Skin = quantitated as 17% body wt.

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## TABLE 36. TISSUE DISTRIBUTION OF BROMODICHLOROMETHANE (AS $^{14}C$) IN MALE RATS

SINGLE ORAL DOSE 20 MG/KG

| | Percent Dose | | µg/g | |
|---|---|---|---|---|
| Tissue | 3 hours | 6 hours | 3 hours | 6 hours |
| Blood | 1.16 | 0.58 | 2.6 | 1.3 |
| Plasma | - | - | 2.1 | 1.9 |
| Liver | 1.8 | 1.4 | 9.0 | 7.3 |
| Spleen | 0.01 | 0.01 | 2.1 | 1.1 |
| Kidney | 0.15 | 0.18 | 4.2 | 4.7 |
| Lungs | 0.03 | 0.02 | 1.5 | 1.00 |
| Heart | 0.02 | 0.01 | 1.60 | 0.97 |
| Testes | 0.03 | 0.02 | 0.82 | 0.46 |
| Brain | 0.02 | 0.01 | 0.89 | 0.54 |
| Fat* | 2.8 | 0.71 | 6.9 | 1.8 |
| Muscle** | 1.8 | 0.62 | 0.91 | 0.31 |
| Skin+ | 0.75 | 0.36 | 0.88 | 0.42 |
| Total Tissue | 8.6 | 3.9 | | |
| Stomach | 21.5 | 35.9 | 284 | 282 |
| Small Intestine | 1.45 | 0.67 | 9.5 | 6.1 |
| Cecum | 0.33 | 0.37 | 3.2 | 5.0 |
| Large Intestine | 0.14 | 0.11 | 1.9 | 2.2 |
| GI Tract & Contents | 23.4 | 37.1 | | |
| **Recovery in Excreta** | | | | |
| Urine | 0.66 | 1.06 | 10.8 | 11.1 |
| Feces | NS | NS | NS | NS |

NS = No sample.
*Fat = quantitated as 8% body wt.
** Muscle = quantitated as 40% body wt.
+Skin = quantitated as 17% body wt.

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was flushed with 20 ml of $H_2O$. The monkeys were housed in metabolism cages maintained within exposure chambers which exhausted all the exhaled compound. The monkeys wore collars with chains that hooked to the cage doors. This facilitated restraining the monkeys to obtain blood samples. They received water ad libitum and were fed 10-15 pellets of monkey chow two hours after treatment and as usual thereafter.

For the two initial studies on each monkey, blood samples were taken before treatment and at 2, 4, 6, 8, 12, 24 and 48 hours. In the two final studies blood samples were obtained at 1, 2, 4, 6, 8, 12 and 24 hours. Any urine lost during blood sampling was caught on a tray over which the monkey was held and recovery of $^{14}C$ in the wash was determined and added to the urine values. Feces were collected if the animal defecated at bleeding times and at 24-hour intervals.

Hematocrits were determined on each blood sample. Whole blood or plasma, urine, urine washes, and feces were analyzed for total $^{14}C$ content employing standard procedures used in this laboratory. Internal standardization was used to correct for quenching.

## Results

Blood levels (as $^{14}C$) after intravenous or oral administration of single 10 mg/kg doses of BDC are shown in Table 37 and Figures 8 and 9. One notes that following intravenous doses the rate falls rapidly ($T_{1/2}$ = ca 4 hr) for the first 8 hours. Then the blood level of $^{14}C$ falls much more slowly for the next 40 hours dropping finally to about 25 μg/ml. The values for No. 3 on 8-19-76 seem abnormally low suggesting poor absorption or possible loss of compound. No loss was noted at the time of treatment and no evidence of abnormally high recovery in urine was detected.

The data following oral doses are more variable but absorption would be judged to be nearly complete except for No. 3 noted above.

Urinary excretion of radioactivity was very low accounting at most for no more than 6% of the dose (Tables 38 and 39 and Figures 10 and 11). Generally, however, excretion was between 2 and 3% and did not appear to change with the route of administration.

Fecal excretion was minimal in every case varying from 0.2 to 1.65% of the dose (Tables 38 and 39). These data, when considered along with the urinary excretion and low tissue concentrations (to be discussed next), indicate that most of the $^{14}C$ from BDC must be exhaled by the lung. The overall similarity of the blood level curves and urinary and fecal excretion curves suggest that again route of administration plays little role in this loss via the lung.

Tissue distribution data in Table 40 point up the small amounts of administered BDC that appear to be sequestered in any tissue. On a μg/g basis one notes that the highest concentrations after an oral dose were found in the bile, cecum, and liver suggesting that some of the $^{14}C$ is excreted via the bile and may not be entirely reabsorbed. Bile, liver, and cecum were highest also after intravenous administration but were not as high as the oral values in these two monkeys. It seems safe to conclude that less than 5% of the administered $^{14}C$ from BDC is present in the body 24 hours after a single oral or intravenous dose of 10 mg/kg.

We can conclude that although monkeys appear to metabolize BDC slightly more extensively than humans metabolize chloroform the compound like chloroform is excreted (exhaled) primarily via the lung. The presence of ca 2.5% of the $^{14}C$ in the urine suggests that some metabolism of BDC occurs but probably accounts for no more than about 5% of the dose. No data are available to indicate whether the $^{14}C$ is exhaled as parent compound or as $CO_2$. In the case of humans, about 50% of the expired $^{13}C$ from stable-labeled chloroform appeared as chloroform and 50% as $^{13}CO_2$.

## IV. MUTAGENICITY TESTS

Urine samples from the 12-24 hr. period for both monkeys 3 and 13 were filtered aseptically and used for mutagenesis assays using the Salmonella typhimurium/mammalian microsome bacterial assay (strains TA98 and TA100) ( 20 ) in the laboratory of Dr. John Loper, Professor of Microbiology, University of Cincinnati, College of Medicine. The filtration through Millipore® filters removed no radioactivity. The analyses indicated that these urine samples exhibited no mutagenic activity toward either bacterial strain.

## TABLE 37. BLOOD CONCENTRATIONS OF BDC (AS $^{14}C$) IN MONKEYS

### SINGLE INTRAVENOUS DOSE OF 10 MG/KG

| Time Post Treatment (Hours) | 3 | | 13 | | 13R | |
|---|---|---|---|---|---|---|
| | Hct | μg/g | Hct | μg/g | Hct | μg/g |
| 1 | -- | -- | -- | -- | 46 | 2.1 |
| 2 | 47 | 1.8 | 42 | 2.1 | 46 | 1.6 |
| 4 | 47 | 1.6 | 41 | 1.7 | 45 | 1.5 |
| 6 | 45 | 1.1 | 42 | 1.2 | 43.5 | 1.1 |
| 8 | 45 | 0.7 | 41 | 0.9 | 43 | 1.0 |
| 12 | 45 | 0.9 | 41 | 0.6 | 44 | 0.7 |
| 24 | 45 | 0.5 | 42 | 0.4 | 40* | 0.4 |
| 48 | 42 | 0.4 | 41 | 0.2 | -- | -- |

*Sacrificed at 24 hours.

### SINGLE ORAL DOSE OF 10 MG/KG

| Time Post Treatment (Hours) | 13 | | 3 | | 3R | |
|---|---|---|---|---|---|---|
| | Hct | μg/g | Hct | μg/g | Hct | μg/g |
| 1 | -- | -- | -- | -- | 48 | 1.2 |
| 2 | 43 | 1.5 | 38 | 0.8 | 46.8 | 1.5 |
| 4 | 43 | 2.2 | 40 | 0.8 | 48 | 2.1 |
| 6 | 42 | 1.7 | -- | 0.5 | 46 | 2.0 |
| 8 | 39 | 0.9 | 39 | 0.3 | 46 | 1.8 |
| 12 | 41 | 0.9 | 39 | 0.2 | 47 | 1.1 |
| 24 | 39 | 0.5 | 40 | 0.1 | 44* | 0.7 |
| 48 | 42 | 0.3 | 38 | <0.1 | -- | -- |

*Sacrificed at 24 hours.

# FIGURE 8

BLOOD CONCENTRATIONS OF BDC (AS $^{14}C$) IN FEMALE RHESUS MONKEYS
SINGLE INTRAVENOUS DOSE 10 MG/KG

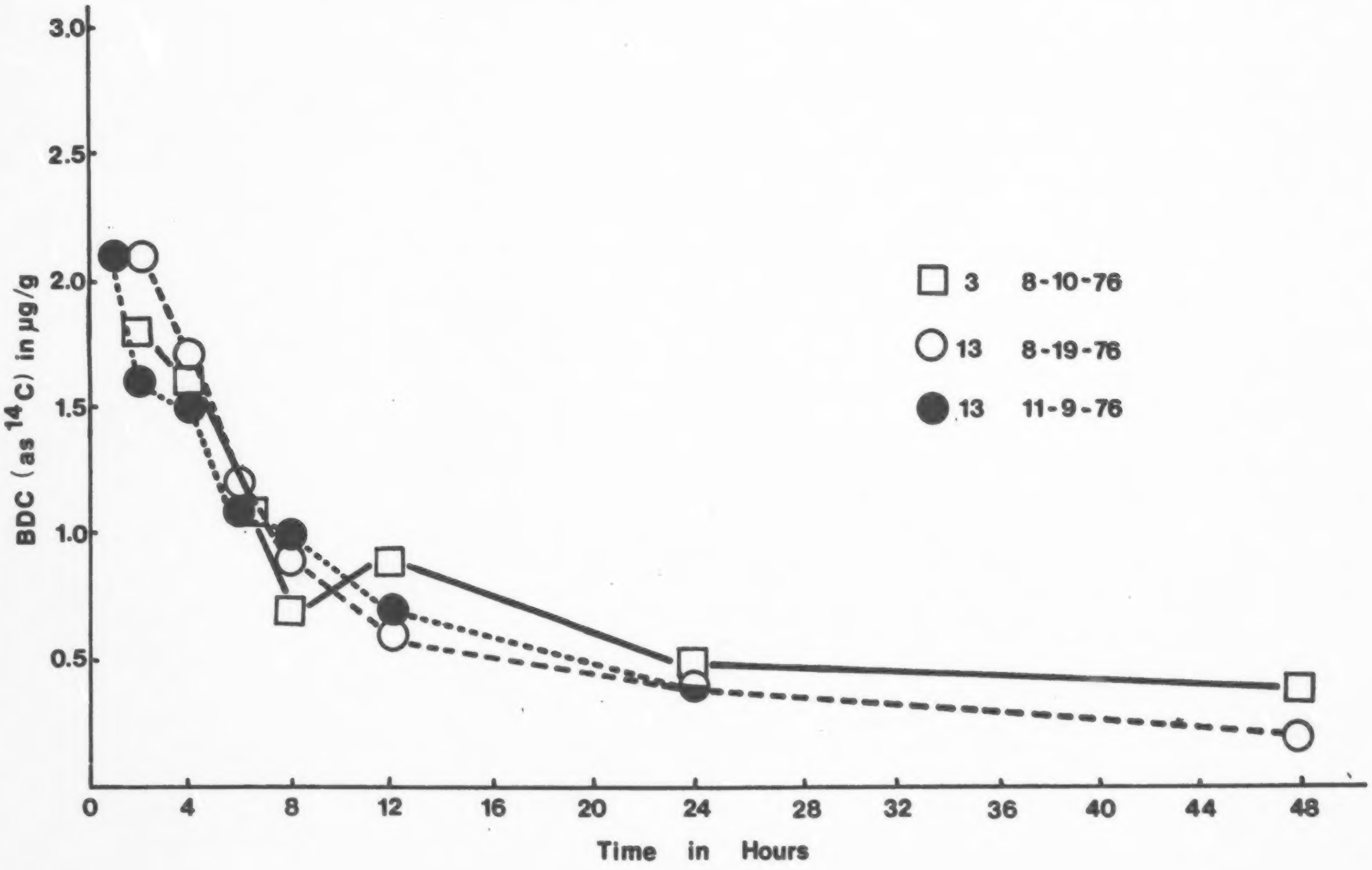


FIGURE 9
BLOOD CONCENTRATIONS OF BDC (AS $^{14}$C) IN FEMALE RHESUS MONKEYS
SINGLE ORAL DOSE 10 MG/KG

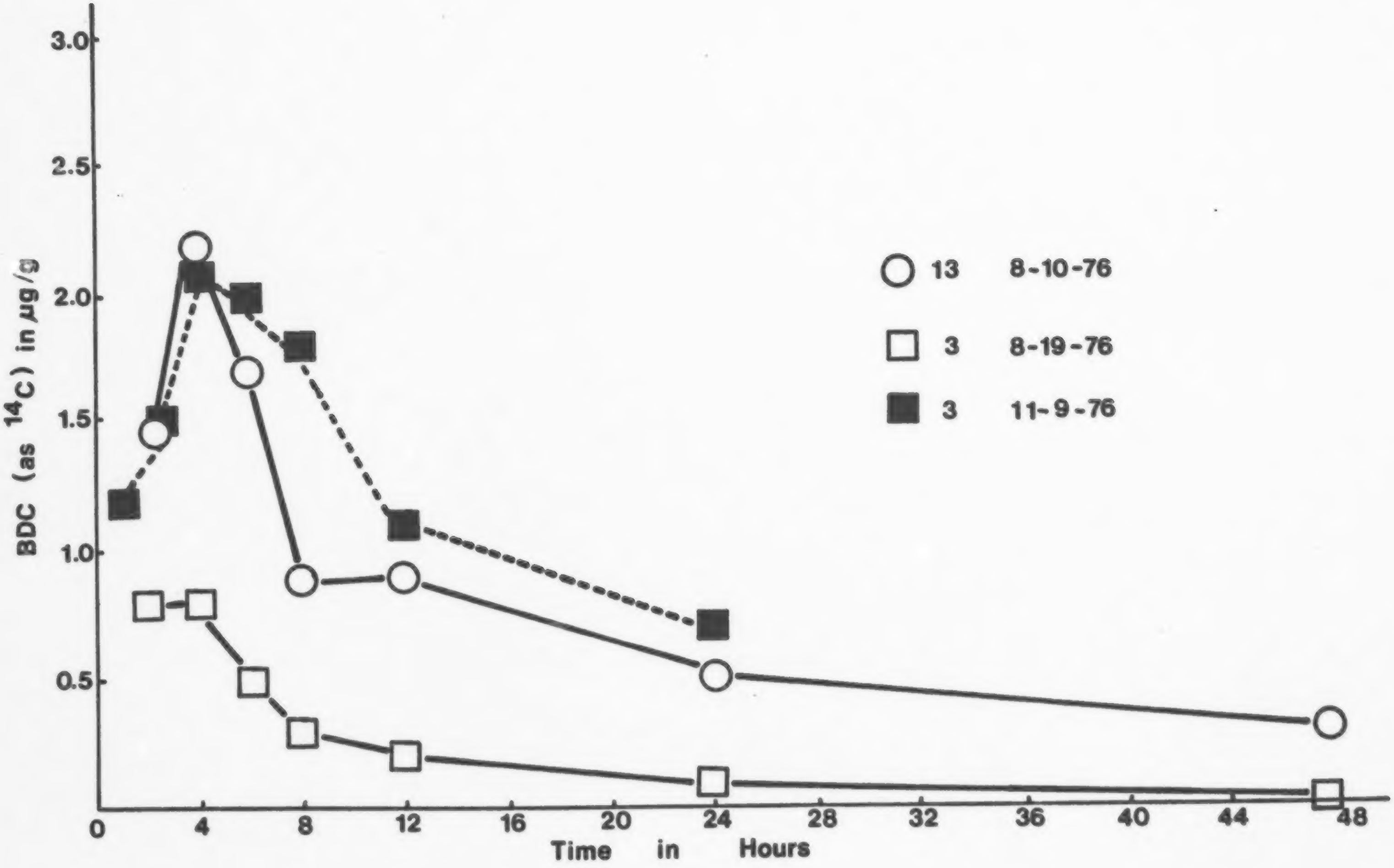


TABLE 38. CUMULATIVE EXCRETION OF BDC (AS $^{14}$C) IN MONKEYS IN % DOSE

SINGLE INTRAVENOUS DOSE 10 MG/KG

| | 3 | | | 13 | | | 13R | | |
|---|---|---|---|---|---|---|---|---|---|
| Hours | Urine | Feces | U & F | Urine | Feces | U & F | Urine | Feces | U & F |
| 1 | -- | -- | -- | -- | -- | -- | <0.01 | -- | <0.01 |
| 2 | -- | -- | -- | -- | -- | -- | 0.14 | 0.02 | 0.16 |
| 4 | 0.29 | -- | 0.29 | 0.51 | 0.04 | 0.55 | 0.29 | 0.06 | 0.35 |
| 6 | -- | -- | -- | -- | -- | -- | 0.38 | -- | 0.44 |
| 8 | -- | -- | -- | -- | -- | -- | 0.52 | -- | 0.58 |
| 12 | 0.64 | -- | 0.64 | -- | -- | -- | 0.87 | 0.09 | 0.96 |
| 24 | 1.54 | 0.20 | 1.74 | 2.11 | 0.15 | 2.26 | *1.78 | 0.48 | 2.26 |
| 48 | 2.10 | -- | 2.30 | 5.99 | 0.21 | 6.20 | -- | -- | -- |

*Sacrificed at 24 hours.

## TABLE 39. CUMULATIVE EXCRETION OF BDC (AS $^{14}C$) IN MONKEYS IN % DOSE

### SINGLE ORAL DOSE 10 MG/KG

| | 13 | | | 3 | | | 3R | | |
|---|---|---|---|---|---|---|---|---|---|
| Hours | Urine | Feces | U & F | Urine | Feces | U & F | Urine | Feces | U & F |
| 1 | -- | -- | -- | -- | -- | -- | <0.01 | -- | <0.01 |
| 2 | -- | -- | -- | -- | -- | -- | 0.04 | -- | 0.04 |
| 4 | 0.10 | -- | 0.10 | 0.10 | 0.03 | 0.13 | 0.23 | -- | 0.23 |
| 6 | -- | -- | -- | 0.16 | 0.05 | 0.21 | 0.72 | -- | 0.72 |
| 8 | -- | -- | -- | -- | -- | -- | 0.94 | -- | 0.94 |
| 12 | -- | -- | -- | 0.20 | -- | 0.25 | 1.08 | -- | 1.08 |
| 24 | 1.19 | 0.21 | 1.40 | 0.75 | 0.24 | 0.99 | *2.61 | 0.06 | 2.67 |
| 48 | 1.88 | 1.52 | 3.40 | 1.49 | 0.48 | 1.97 | -- | -- | -- |
| 72 | | 1.65 | 3.53 | -- | -- | -- | -- | -- | -- |

*Sacrificed at 24 hours.

FIGURE 10
CUMULATIVE URINE EXCRETION OF BDC IN MONKEYS
SINGLE INTRAVENOUS DOSE 10 MG/KG

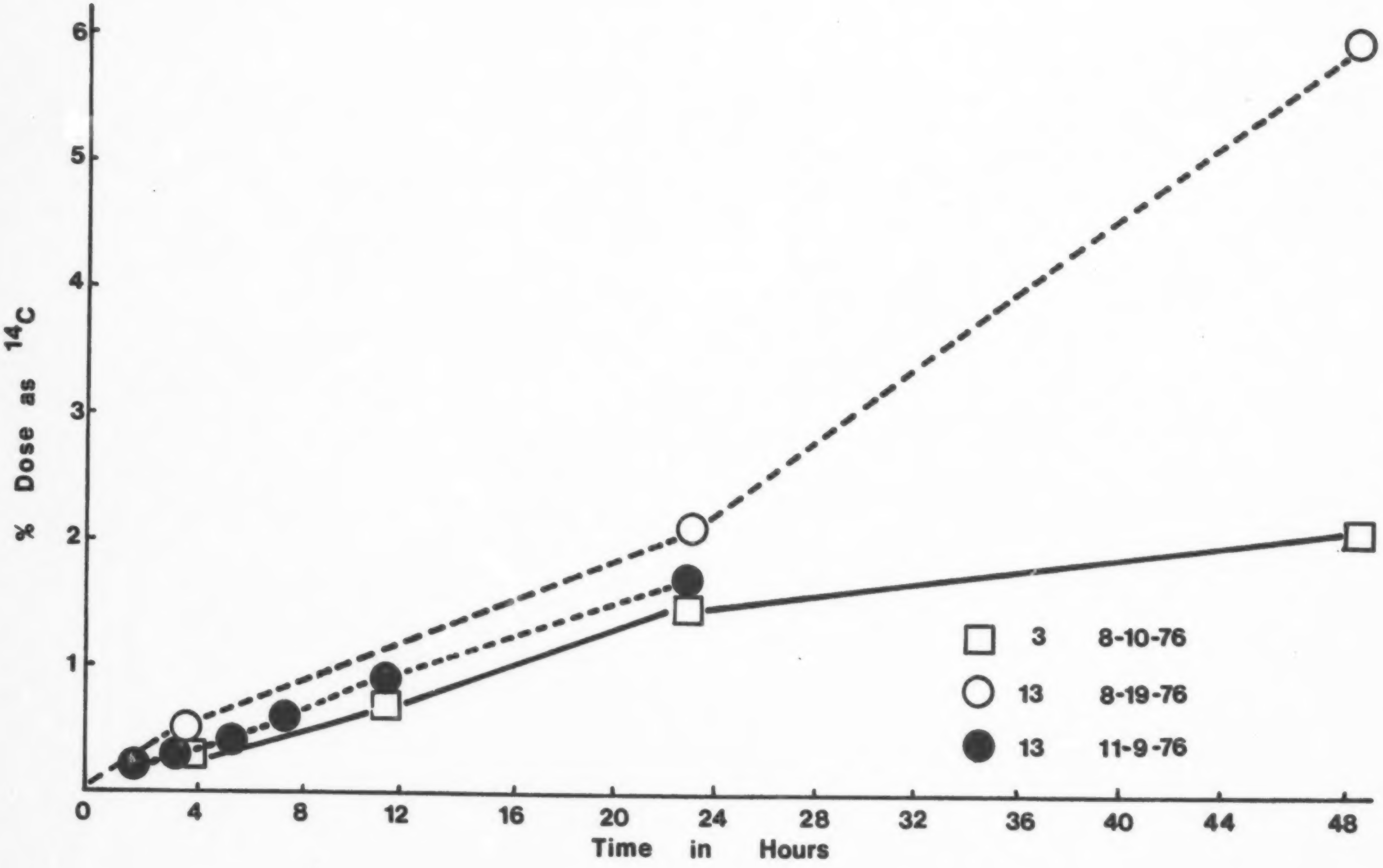
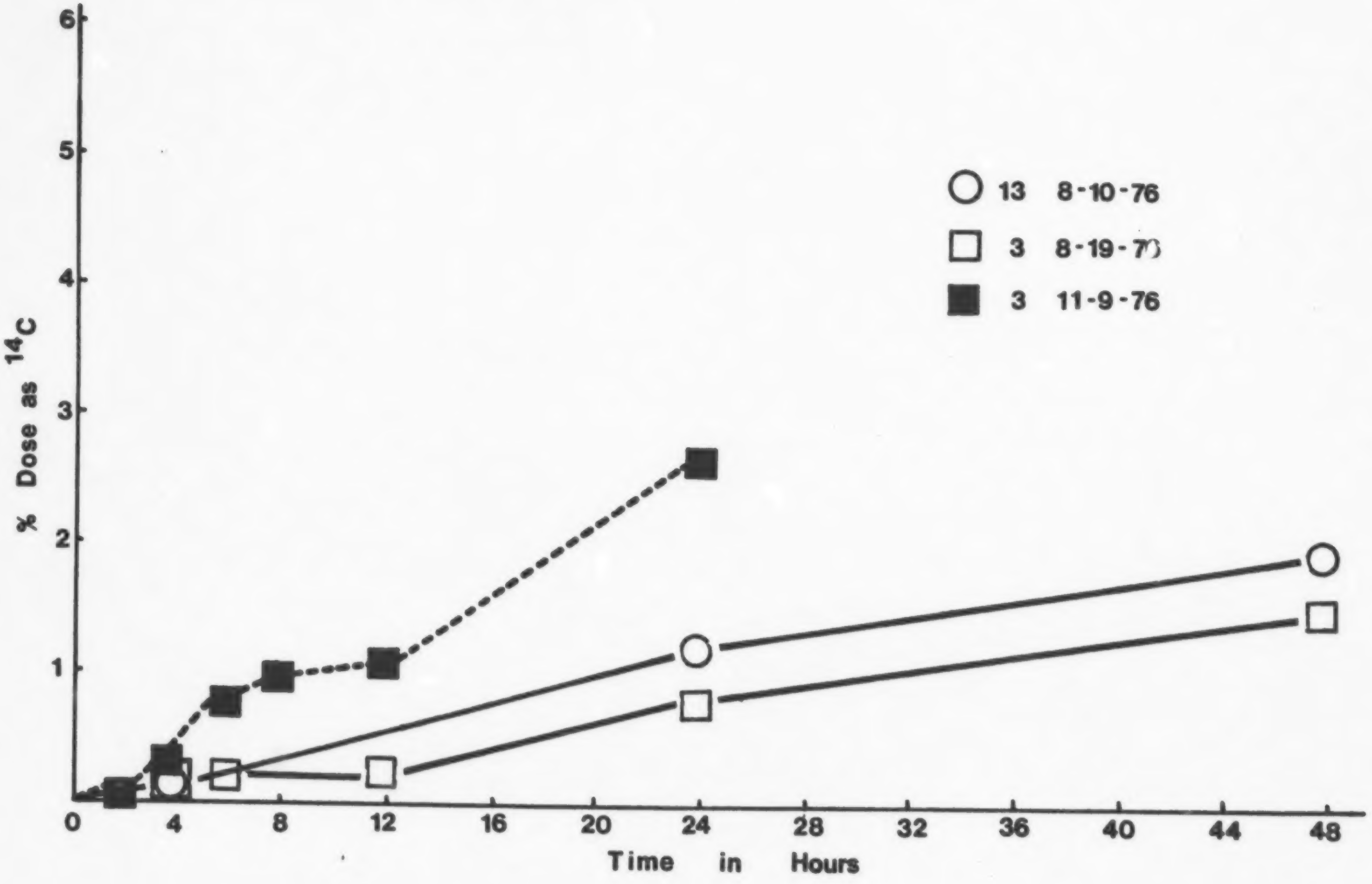


FIGURE 11
CUMULATIVE URINE EXCRETION OF BDC IN MONKEYS
SINGLE ORAL DOSE OF 10 MG/KG



## TABLE 40. TISSUE DISTRIBUTION OF BDC (AS $^{14}C$) IN MONKEYS AT 24 HOURS

SINGLE DOSE OF 10 MG/KG

| Tissue | 3R Oral | 13R I.V. | 3R Oral | 13R I.V. |
|---|---|---|---|---|
| | % of Dose | | µg/g | |
| Blood | 0.66 | 0.41 | 0.65 | 0.43 |
| Plasma | -- | -- | 0.99 | 0.63 |
| Liver | 2.38 | 1.84 | 11.2 | 9.0 |
| Gall Bladder | 0.01 | <0.01 | 4.1 | 2.3 |
| Bile | 0.06 | 0.03 | 32.0 | 16.2 |
| Pancreas | 0.02 | 0.01 | 1.1 | 0.5 |
| Spleen | 0.01 | 0.01 | 0.9 | 0.6 |
| Kidney | 0.05 | 0.02 | 1.4 | 0.5 |
| Adrenals | <0.01 | <0.01 | 1.5 | 1.0 |
| Lungs | 0.04 | 0.03 | 0.8 | 0.5 |
| Heart | 0.02 | 0.01 | 0.7 | 0.4 |
| Skeletal Muscle † | 0.97 | 0.42 | 0.5 | 0.2 |
| Abdominal Muscle †† | 0.50 | 0.22 | 0.5 | 0.2 |
| Skin ††† | <0.01 | <0.01 | 0.6 | 0.3 |
| Fat †††† | 0.48 | 1.0 | 0.6 | 1.3 |
| Stomach* | 0.05 | 0.05 | 0.9 | 0.7 |
| Small Intestine* | 0.17 | 0.13 | 1.7 | 1.1 |
| Cecum* | 0.29 | 0.70 | 15.4 | 4.6 |
| Large Intestine* | 1.39 | 0.58 | 9.6 | 2.2 |
| Ovaries | <0.01 | <0.01 | 1.1 | 0.6 |
| Sex Tract** | 0.02 | 0.02 | 0.7 | 0.4 |
| Urinary Bladder | 0.01 | 0.03 | 0.5 | 0.5 |
| Brain | 0.07 | 0.04 | 0.5 | 0.3 |
| Eyes | 0.01 | <0.01 | 0.2 | 0.2 |
| Bone Marrow | 0.01 | 0.01 | 3.0 | 3.7 |

† Skeletal muscle calculated as 20% of body weight.

†† Abdominal muscle calculated as 10% body weight.

††† %/g or µg/g.

†††† Fat calculated as 8% body weight.

* Tissue plus contents.

** Uterus, vagina and fallopian tubes.

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# SECTION 7

## STUDIES ON BIS (2-CHLOROISOPROPYL ETHER)

### I. PHARMACOKINETICS OF BIS(2-CHLOROISOPROPYL) ETHER-$^{14}C$ IN RHESUS MONKEYS FOLLOWING SINGLE INTRAVENOUS OR ORAL DOSES

### Introduction

Bis(2-chloroisopropyl) ether (BCIE) is one of several halogenated organic compounds which have been detected in potable water supplies. Our early preliminary studies in rats and monkeys were published in 1977(21).More recently we have extended these studies to include additional observations in monkeys given both single and multiple doses.

### SINGLE DOSE STUDIES OF BCIE IN MONKEYS

A total of seven female rhesus monkeys have been used in defining the single dose toxicity of BCIE. Three were dosed intravenously and four orally and sacrificed as follows:

Intravenous dose-after 4, 168, and 168 hours

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Oral dose-after 4, 6, 168, and 168 hours

### Materials and Methods

The treatment solution for intravenous administration was prepared by adding 1.3 ml of bis(2-chlorodiisopropyl) ether and 200 µl of bis(2-chlorodiisopropyl) ether-$^{14}C$ (0.5 mCi/ml) to 15 ml of Emulphor® (EL-620):ethanol (100%) (1:1, v/v) and diluted with deionized distilled water to 50 ml. The resulting solution contained 33.1 mg/ml and 1.32 µCi/ml with a specific activity of 88.68 dpm/µg, established by replicate assays of the treatment solution.

The oral treatment solution was prepared by adding 20 ml of the intravenous solution described above to 20 ml of Emulphor®-ethanol (1:1, v/v) q.s. to 100 ml with $H_2O$. The resulting solution contained 6.62 mg/ml and 0.27 µCi per ml with specific activity of 91.96 dpm/µg. The latter value was established by replicate assays of the oral treatment solution.

Three female rhesus monkeys were injected with BCIE in single intravenous doses of 30 mg/kg. One monkey, 295 I (4.8 kg), was injected via the cephalic vein of the left forearm; two monkeys 138 (5.6 kg) and 100 (5.7 kg), were injected via the saphenous vein of the left leg. Four female monkeys, weighing 2.7 to 4.6 kg, were given the same dose of the compound by gastric intubation using a rubber catheter. After the dose was given the catheter was rinsed by administering 20 ml of water.

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The monkeys were housed in metabolism cages maintained within exposure chambers which exhausted all the exhaled compound. The monkeys wore collars with chains that hooked to the cage doors. This facilitated obtaining blood samples. They received water ad libitum and were fed 10-15 pellets of monkey chow two hours after treatment and as usual thereafter.

Blood samples were taken before treatment and at 2, 4, 6, 8, 12, 24, 48 72, 96, 120, 144, 168 hours on the monkeys sacrificed at seven days. For those sacrificed at 4 and 6 hr, blood samples were taken at 1, 2, 4 and 6 hr. Urine samples when available were collected at 4, 6, 8, 12, 24, 48, 72, 96, 120, 144, and 168 hours for the seven day studies. In addition, any urine lost during bleeding was caught on a tray over which the monkey was held. Recovery of $^{14}$C in the tray wash was determined and added to the urine figures. Feces were collected at 24 hour intervals in the seven day studies, and whenever available in the short-term studies.

Hematocrits were determined on each blood sample and whole blood, urine, feces, bile and tissues were analyzed for total $^{14}$C content employing the standard procedures used in this laboratory, Internal standardization was used to correct for quenching.

## Results

When this compound was administered intravenously, there was a rapid drop from an estimated zero level of about 330 μg/g to 32.7 μg/g at two hours post-treatment in 295 I (Table 41 and Figure 12). In monkey 100 the level dropped from the estimated zero value to 30.3 μg/g at 1 hr and 24.3 μg/g at 2 hrs. The level for both animals at 12 hr was 8.4 μg/g, then the values dropped slowly to about 3 μg/g at 96 hours and about 2.5 μg/g (as $^{14}$C) at 168 hours. Plasma levels of BCIE and metabolites were slightly higher than values for whole blood up to 120 hours but after that time, the values for whole blood and plasma were about equal (Table 42).

The orally treated monkeys studied for seven days showed peak blood levels at 2 hr after treatment, the short-term monkeys at 2 hr and at 4-6 hr (Table 43 and Figure 13). The 2 hr blood levels in the 4 monkeys ranged from 20.1 to 26.1 μg/g and the 4 hr levels ranged from 16.8 - 24.5 μg/g. As was observed above in the I.V. monkeys the blood levels dropped slowly to an average value of 2.4 μg/g at 24 hours and to about 1.5 μg/g at 7 days. There was no real difference in the $^{14}$C levels in whole blood and plasma indicating that BCIE and/or its metabolites are not strongly excluded from or sequestered in the red cell (Table 44).

The two seven day monkeys on intravenous treatment excreted 35 and 29% of their dose in the urine (Table 45 and Figure 14). Four hour cumulative urinary excretion varied from 13.9 to 23% representing about 50% of the total excreted in the urine. Thus the drug was rapidly eliminated by the kidneys but total urinary excretion accounted for only a small part of the total dose administered.

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Fecal excretion over the seven day period amounted to only 1.2 to 1.56% of the dose (Table 45). From this one can conclude that excretion in urine and feces accounted for only about one-third of the administered dose when the compound was injected intravenously.

When the compound was administered orally total urinary excretion after 168 hours accounted for 24.1 and 30.1% of the dose (Table 46 and Figure 15). About 50% of the total urinary excretion occurred by 8 hours. Total fecal excretion amounted to 1.59 to 1.91% of the dose (Table 46), values very similar to those observed when BCIE was injected intravenously. Thus we can conclude that following either oral or intravenous administration of labeled BCIE only a third or less is recovered in the urine and feces. Very little additional radioactivity was recovered in the tissues as noted below.

Data on tissue distribution of BCIE and metabolites in 1 monkey was included in the manuscript entitled "Comparative Metabolism of Haloethers", a copy of which is attached. Our additional data on tissue distribution in this report (Tables 47, 48, 49, 50) suggest that the only organs showing significant uptake of $^{14}$C from BCIE are the liver and kidneys. All the other tissues showed lower concentrations of $^{14}$C which were about equal to the levels in blood. Fat has slight sequestering activity but always less than liver. There appears to be some biliary excretion of BCIE and/or its metabolites but at 7 days the concentration or total amount of $^{14}$C in bile is not remarkable. Although no data for 24 hours are available it can be deduced that there is very little sequestration of the compound or its metabolites in any tissue except that observed in liver, fat and skeletal muscle. The total in these three organs, at the end of 7 days, is only about 4% of the administered dose (of $^{14}$C).

## II. MULTIPLE DOSE STUDY OF BIS(2-CHLOROISOPROPYL)ETHER IN MONKEYS

The purpose of this experiment was to determine the effect of multiple oral daily doses of bis-(2-chloroisopropyl) ether (BCIE) in female rhesus monkeys. In particular, we wished to examine the pharmacokinetics of BCIE following multiple oral doses.

### Materials and Methods

The BCIE treatment solution was prepared from $^{14}$C labeled BCIE and stable BCIE as follows: to 43 ml of Emulphor® and 40 ml of absolute ethanol was added 2.90 ml or 3200 mg of cold BCIE and enough $^{14}$C labeled BCIE was added to provide, when diluted with water, an oral treatment solution of 400 ml containing 8 mg BCIE and 0.21 $\mu$Ci of $^{14}$C per ml of treatment solution.

Three female rhesus monkeys weighing 6.4 to 7.4 kg were used. They were housed individually in primate metabolism cages placed within exposure chambers which exhausted all the exhaled compound. The monkeys wore collars with chains which hooked on the doors of the metabolism cages. This facilitated restraining the monkey for bleeding. They were fed water *ad libitum* and monkey chow *ad libitum* beginning 2 hours after each oral dose.

## TABLE 41. BLOOD CONCENTRATIONS OF BCIE (AS $^{14}C$) IN MONKEYS

SINGLE INTRAVENOUS DOSE 30 MG/KG

| Time Post Treatment Hours | 138♀ 4 Hr % Hct | 138♀ 4 Hr µg/g | 295 I♀ 168 Hr % Hct | 295 I♀ 168 Hr µg/g | 100♀ 168 Hr % Hct | 100♀ 168 Hr µg/g |
|---|---|---|---|---|---|---|
| 1 | 39.3 | 30.6 | - | | 39.5 | 30.3 |
| 2 | 36.8 | 25.9 | 41 | 32.7 | 40.0 | 24.3 |
| 4 | 35.5 | 19.0 | 42 | 24.5 | 38.0 | 17.8 |
| 6 | | | 42 | 20.7 | 39.0 | 15.0 |
| 8 | | | 43 | 15.2 | 38.0 | 11.3 |
| 12 | | | 35 | 8.4 | 38.0 | 8.4 |
| 24 | | | 40 | 4.4 | 37.0 | 5.1 |
| 48 | | | 39 | 4.2 | 36.0 | 4.0 |
| 72 | | | 37 | 2.7 | 37.5 | 3.5 |
| 96 | | | 38 | 2.9 | 38.0 | 3.4 |
| 120 | | | 38 | 2.7 | 37.0 | 2.9 |
| 144 | | | 36 | 2.9 | 37.5 | 2.8 |
| 168 | | | 35 | 2.4 | 37.5 | 2.5 |

FIGURE 12
BLOOD LEVELS OF BCIE IN MONKEYS
SINGLE INTRAVENOUS DOSE 30 MG/KG

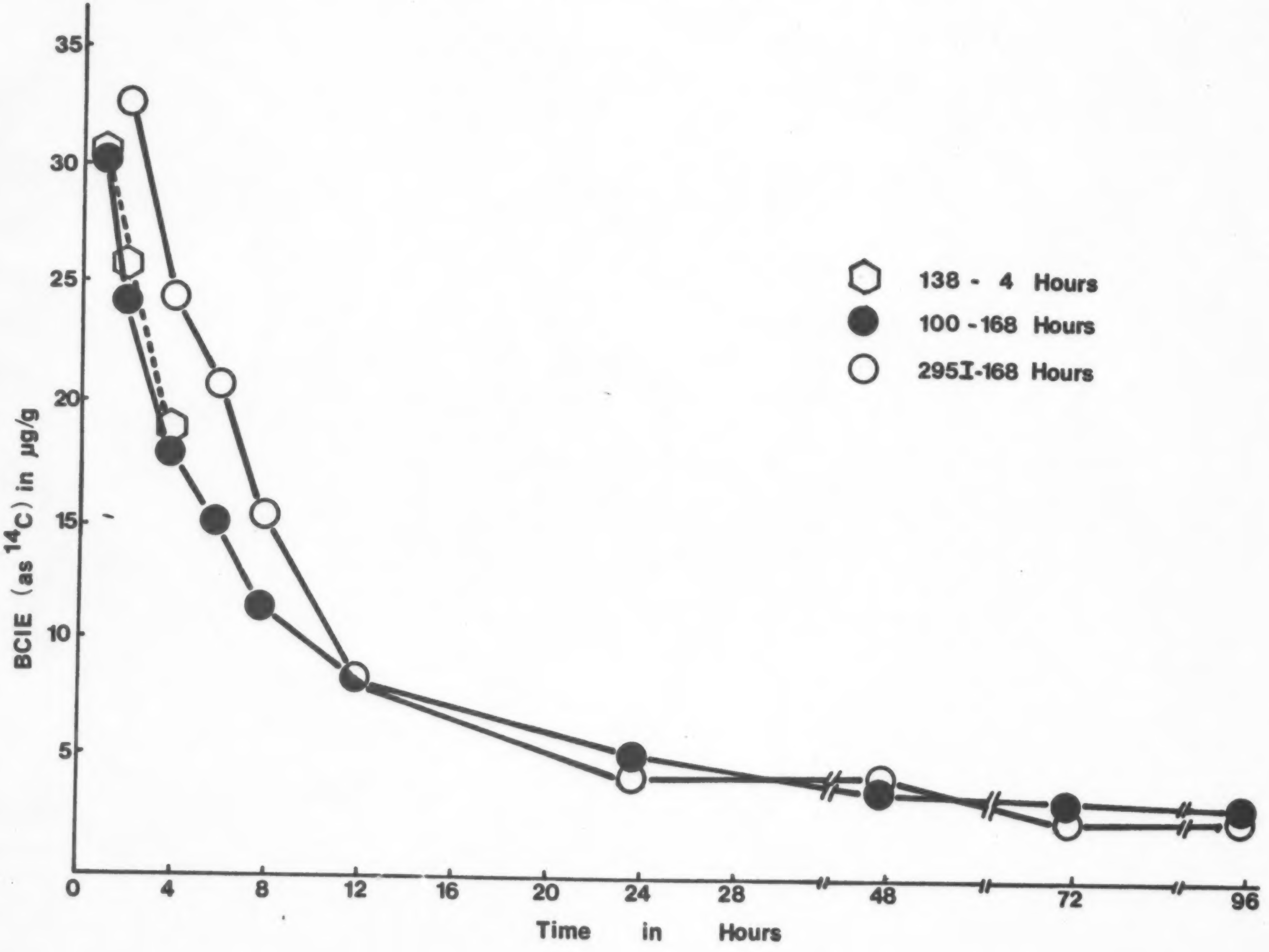

TABLE 42. PLASMA CONCENTRATIONS OF BCIE (AS $^{14}C$) IN MONKEYS

SINGLE INTRAVENOUS DOSE OF 30 MG/KG

| Time Post Treatment Hours | 138♀ 4 Hr µg/g | 295 I♀ 168 Hr µg/g | 100♀ 168 Hr µg/g |
|---|---|---|---|
| 1 | 33.2 | | 32.5 |
| 2 | 27.3 | 35.0 | 28.1 |
| 4 | 19.4 | 27.2 | 19.8 |
| 6 | | 21.6 | 17.7 |
| 8 | | 18.0 | 14.4 |
| 12 | | 10.1 | 11.7 |
| 24 | | 6.2 | 6.8 |
| 48 | | 5.0 | 4.7 |
| 72 | | 3.2 | 3.9 |
| 96 | | 3.0 | 3.5 |
| 120 | | 2.6 | 2.8 |
| 144 | | 2.7 | 2.4 |
| 168 | | 2.4 | 2.1 |

## TABLE 43. BLOOD CONCENTRATIONS OF BCIE (AS $^{14}C$) IN MONKEYS

### SINGLE ORAL DOSE 30 MG/KG

| Time Post Treatment | 111♀ 4 Hr | | 116♀ 6 Hr | | 290 F♀ 168 Hr | | 75-6♀ 168 Hr | |
|---|---|---|---|---|---|---|---|---|
| Hours | % Hct | µg/g | % Hct | µg/g | % Hct | µg/g | % Hct | µg/g |
| 1 | 37.8 | 18.9 | 28 | 12.3 | - | - | - | - |
| 2 | 37.5 | 26.1 | 29 | 20.1 | 46 | 21.3 | 45 | 25.1 |
| 4 | 36.0 | 23.4 | 28 | 24.5 | 44 | 16.8 | - | 17.8 |
| 6 | | | 28.3 | 24.4 | 42 | 18.4 | 45 | 13.1 |
| 8 | | | | | 40 | 13.1 | 44 | 6.7 |
| 12 | | | | | 40 | 6.9 | 43 | 2.5 |
| 24 | | | | | 39 | 3.5 | 45 | 1.3 |
| 48 | | | | | 40 | 3.1 | 42 | 1.6 |
| 72 | | | | | 39 | 2.3 | 42 | 1.5 |
| 96 | | | | | 41 | 2.2 | 42 | 1.3 |
| 120 | | | | | 40.5 | 2.3 | 43.5 | 1.3 |
| 144 | | | | | 40 | 2.2 | 43 | 1.3 |
| 168 | | | | | 39 | 1.9 | 43 | 1.2 |

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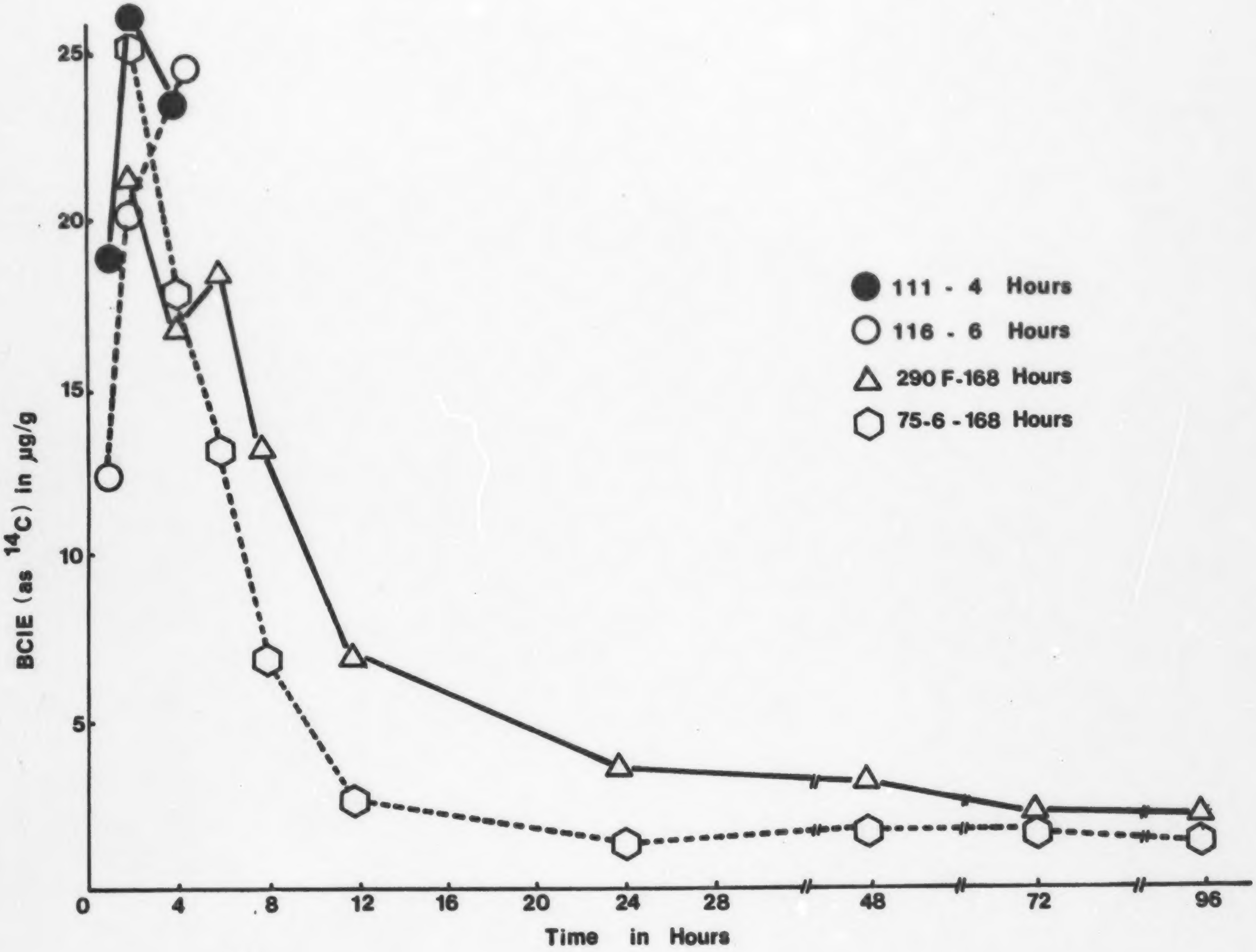
## FIGURE 13
## BLOOD LEVELS OF BCIE IN MONKEYS
## SINGLE ORAL DOSE 30 MG/KG



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## TABLE 44. PLASMA CONCENTRATIONS OF BCIE (AS $^{14}C$) IN MONKEYS

SINGLE ORAL DOSE OF 30 MG/KG

| Time Post Treatment Hours | 111♀ 4 Hr µg/g | 116♀ 6 Hr µg/g | 290F♀ 168 Hr µg/g | 75-6♀ 168 Hr µg/g |
|---|---|---|---|---|
| 1 | 20.3 | 12.4 | | |
| 2 | 27.0 | 21.7 | 23.8 | 27.9 |
| 4 | 23.1 | 23.0 | 17.2 | - |
| 6 | | | 18.7 | 12.6 |
| 8 | | | 13.0 | 6.9 |
| 12 | | | 9.2 | - |
| 24 | | | 4.7 | 1.9 |
| 48 | | | 3.4 | 1.5 |
| 72 | | | 2.6 | 1.3 |
| 96 | | | 1.9 | 1.3 |
| 120 | | | 2.2 | 1.0 |
| 144 | | | 1.9 | 0.9 |
| 168 | | | 1.8 | 0.7 |

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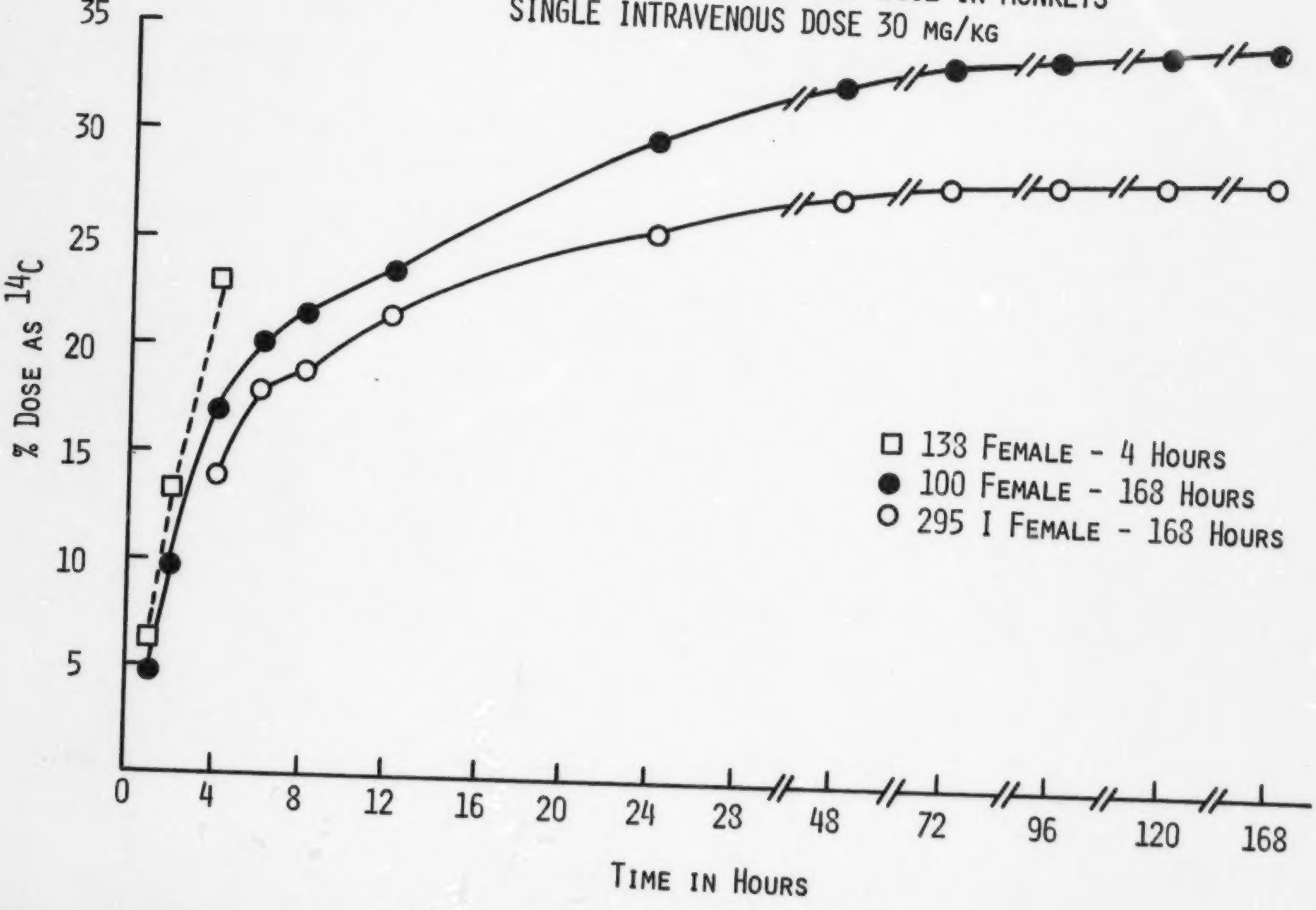
TABLE 45. CUMULATIVE EXCRETION OF BCIE (AS $^{14}C$) IN MONKEYS

SINGLE INTRAVENOUS DOSE OF 30 MG/KG

| Time Post Treatment (Hours) | Percent Dose<br>138<br>4 hr<br>Urine | <br><br><br>Feces | <br><br><br>U & F | <br>100<br>168 hr<br>Urine | <br><br><br>Feces | <br><br><br>U & F | <br>295 I<br>168 hr<br>Urine | <br><br><br>Feces | <br><br><br>U & F |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.22 | -- | 6.22 | 4.68 | -- | 4.68 | -- | -- | -- |
| 2 | 13.21 | -- | 13.21 | 9.61 | -- | 9.61 | -- | -- | -- |
| 4 | 23.01 | 0.01 | 23.02* | 16.85 | <0.01 | 16.85 | 13.88 | 0.01 | 13.89 |
| 6 | | | | 20.02 | <0.01 | 20.02 | 17.84 | 0.01 | 17.85 |
| 8 | | | | 21.48 | -- | 21.48 | 18.71 | -- | 18.72 |
| 12 | | | | 23.51 | -- | 23.51 | 21.40 | -- | 21.41 |
| 24 | | | | 29.81 | 0.44 | 30.25 | 25.49 | 0.02 | 25.51 |
| 48 | | | | 32.49 | 0.97 | 33.46 | 27.33 | 0.08 | 27.41 |
| 72 | | | | 33.53 | 1.27 | 34.80 | 27.98 | 0.88 | 28.86 |
| 96 | | | | 34.03 | 1.36 | 35.39 | 28.24 | 1.05 | 29.29 |
| 120 | | | | 34.37 | 1.45 | 35.82 | 28.40 | 1.13 | 29.53 |
| 144 | | | | 34.59 | 1.52 | 36.11 | 28.52 | 1.18 | 29.70 |
| 168 | | | | 34.76 | 1.56 | 36.32* | 28.62 | 1.20 | 29.82* |

*Sacrificed

FIGURE 14
CUMULATIVE URINARY EXCRETION OF BCIE IN MONKEYS
SINGLE INTRAVENOUS DOSE 30 MG/KG



## TABLE 46. CUMULATIVE EXCRETION OF BCIE (AS $^{14}C$) IN MONKEYS

SINGLE ORAL DOSE OF 30 MG/KG

| | Percent Dose | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Time Post | | 111 | | | 116 | | | 290 F | | | 75-6 | |
| Treatment | | 4 hr | | | 6 hr | | | 168 hr | | | 168 hr | |
| (Hours) | Urine | Feces | U & F | Urine | Feces | U & F | Urine | Feces | U & F | Urine | Feces | U & F |
| 1 | 0.96 | -- | 0.96 | 0.46 | -- | 0.46 | -- | -- | -- | -- | -- | -- |
| 2 | 2.91 | -- | 2.91 | 1.21 | -- | 1.21 | -- | -- | -- | -- | -- | -- |
| 4 | 14.20 | 1.55 | 15.75* | 7.69 | -- | 7.69 | 7.91 | -- | 7.91 | 13.19 | 0.01 | 13.20 |
| 6 | | | | 13.69 | 1.42 | 15.11* | 9.22 | 0.01 | 9.23 | 13.72 | -- | 13.73 |
| 8 | | | | | | | 12.22 | -- | 12.23 | 14.10 | 0.08 | 14.18 |
| 12 | | | | | | | 16.49 | -- | 16.50 | 26.22 | -- | 26.30 |
| 24 | | | | | | | 19.43 | 0.30 | 19.73 | 28.56 | 1.08 | 29.64 |
| 48 | | | | | | | 22.95 | 0.39 | 23.34 | 29.42 | 1.21 | 30.63 |
| 72 | | | | | | | 23.35 | NS | 23.74 | 29.70 | 1.39 | 31.09 |
| 96 | | | | | | | 23.64 | 0.46 | 24.10 | 29.88 | 1.49 | 31.37 |
| 120 | | | | | | | 23.85 | 1.46 | 25.31 | 29.97 | 1.52 | 31.49 |
| 144 | | | | | | | 23.95 | 1.66 | 25.61 | 30.03 | 1.55 | 31.58 |
| 168 | | | | | | | 24.05 | 1.91 | 25.96* | 30.09 | 1.59 | 31.68* |

*Sacrificed

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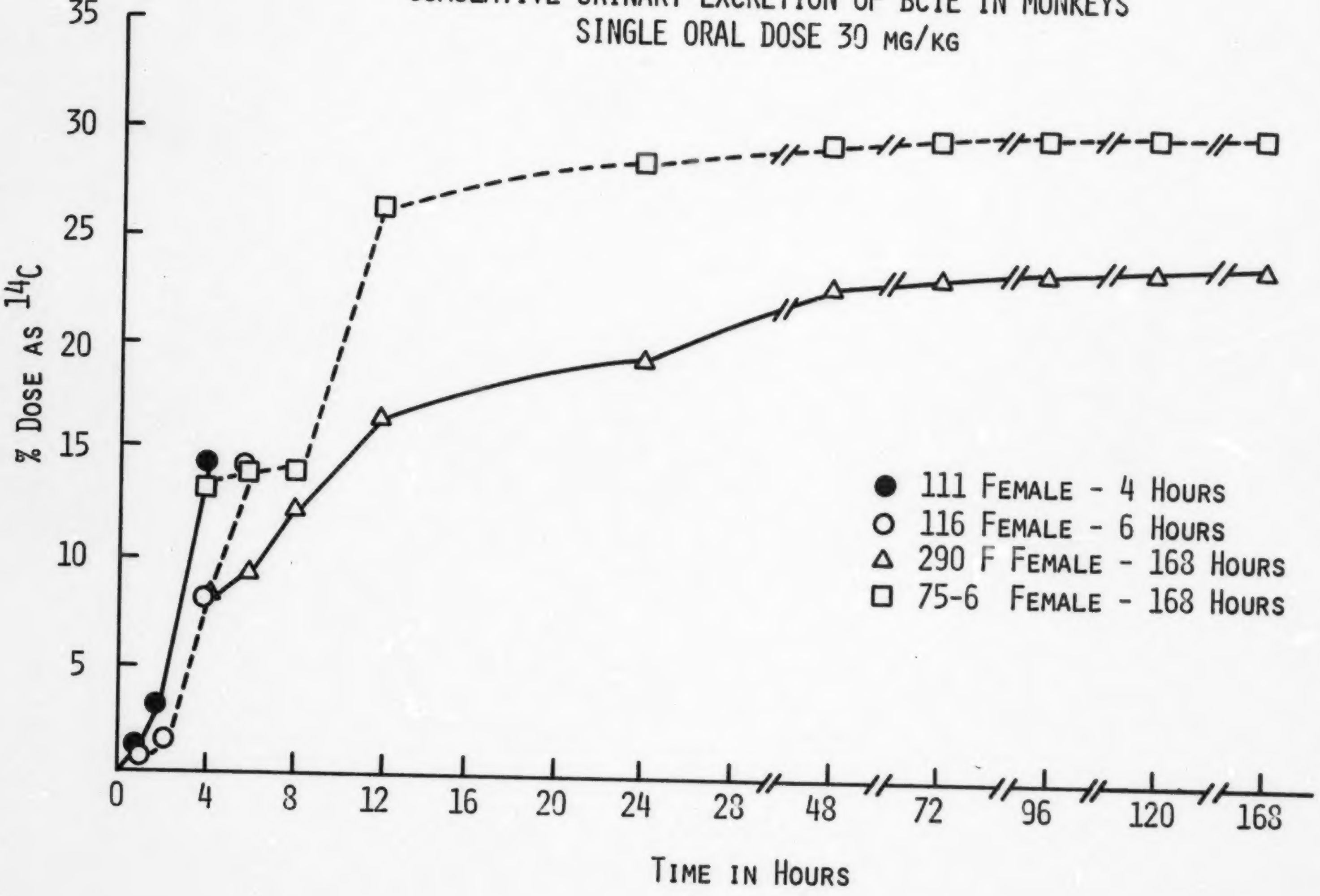
FIGURE 15
CUMULATIVE URINARY EXCRETION OF BCIE IN MONKEYS
SINGLE ORAL DOSE 30 MG/KG



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## TABLE 47. TISSUE DISTRIBUTION OF BCIE (AS $^{14}$C) IN MONKEYS

### SINGLE INTRAVENOUS DOSE OF 30 MG/KG

| | µg/g | | |
|---|---|---|---|
| Tissue | 138♀<br>4 Hr | 295I♀<br>168 Hr | 100♀<br>168 Hr |
| Blood | 19.0 | 2,4 | 2.5 |
| Plasma | 19.4 | 2.4 | 2.1 |
| Liver | 73.8 | 28.8 | 17.2 |
| Gall Bladder | 64.2 | 5.0 | 1.8 |
| Bile | 98.9 | 6.0 | 9.0 |
| Pancreas | 26.7 | 3.8 | 3.1 |
| Spleen | 23.3 | 4.5 | 10.5 |
| Kidneys | 48.6 | 5.0 | 5.2 |
| Adrenals | 24.9 | 6.6 | 8.9 |
| Lungs | 18.2 | 3.0 | 3.1 |
| Heart | 20.0 | 3.0 | 2.6 |
| Skel M. | 12.5 | 3.3 | 2.0 |
| Abdom M. | 10.0 | 2.4 | 3.3 |
| Skin | 17.1 | 3.0 | 3.8 |
| Fat | 26.4 | 3.2 | 4.5 |
| Stomach* | 17.9 | 2.6 | 1.5 |
| Small Int.* | 38.6 | 3.5 | 4.1 |
| Cecum* | 73.1 | 1.8 | 2.6 |
| Lg. Int.* | 16.7 | 2.1 | 2.6 |
| Ovaries | 15.6 | 2.3 | 3.6 |
| Sex T.** | 17.7 | 1.95 | 3.7 |
| U. Bladder | *** | 1.8 | 1.5 |
| U. Bl. Urine | - | 0.5 | 2.5 |
| Brain | 20.1 | 3.3 | 2.4 |
| Eyes | 13.0 | 0.3 | 0.5 |
| Bone Marrow | 24.6 | 3.3 | 3.7 |
| Aorta | 11.8 | 1.8 | - |
| Orbital Fat | - | - | - |
| Cardiac Fat | - | 3.0 | 3.4 |
| Mesenteric Fat | 26.2 | - | - |

* Tissue plus contents.

** Uterus, vagina and fallopian tubes.

*** No sample.

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## TABLE 48. TISSUE DISTRIBUTION OF BCIE (AS $^{14}C$) IN MONKEYS

SINGLE INTRAVENOUS DOSE OF 30 MG/KG

|  | % Dose Administered |  |  |
|---|---|---|---|
| Tissue | 138♀<br>4 Hr | 2951♀<br>168 Hr | 100♀<br>168 Hr |
| Blood | 5.76 | 0.74 | 0.75 |
| Liver | 4.83 | 1.34 | 1.0 |
| Gall Bladder | 0.04 | <0.01 | <0.01 |
| Bile | 0.05 | 0.01 | 0.02 |
| Pancreas | 0.10 | 0.02 | 0.01 |
| Spleen | 0.09 | 0.01 | 0.02 |
| Kidneys | 0.61 | 0.05 | 0.04 |
| Adrenals | 0.02 | <0.01 | <0.01 |
| Lungs | 0.42 | 0.05 | 0.04 |
| Heart | 0.24 | 0.03 | 0.02 |
| Skel M.† | 7.58 | 2.01 | 1.17 |
| Abdom M.†† | 3.04 | 0.75 | 0.96 |
| Skin ††† | 0.01 | <0.01 | <0.01 |
| Fat †††† | 6.40 | 0.78 | 1.05 |
| Stomach* | 0.37 | 0.05 | 0.14 |
| Small Int.* | 1.46 | 0.11 | 0.14 |
| Cecum* | 1.27 | 0.03 | 0.05 |
| Large Int.* | 0.49 | 0.09 | 0.10 |
| Ovaries | 0.01 | <0.01 | <0.01 |
| Sex T.** | 0.31 | 0.01 | 0.02 |
| U. Bladder | N.S. | 0.01 | <0.01 |
| U. Bl. Urine | - | <0.01 | <0.01 |
| Brain | 1.00 | 0.18 | 0.11 |
| Eyes | 0.06 | <0.01 | <0.01 |
| Bone M. | 0.01 | <0.01 | <0.01 |
| Aorta | 0.01 | <0.01 | - |
| Eye Fat | - | - | - |
| Heart Fat | - | <0.01 | <0.01 |
| Mesenteric Fat* | 0.01 | - |  |

† Skeletal muscle, calculated as 20% body weight.

†† Abdominal muscle, calculated as 10% body weight.

††† %/g or μg/g.

†††† Fat calculated as 8% body weight.

* Tissue plus contents.

** Uterus, vagina and fallopian tubes.

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## TABLE 49. TISSUE DISTRIBUTION OF BCIE (AS $^{14}C$) IN MONKEYS

SINGLE ORAL DOSE OF 30 MG/KG

| | µg/g | | | |
|---|---|---|---|---|
| | 111♀ | 116♀ | 290F ♀ | 75-6♀ |
| Tissue | 4 Hr | 6 Hr | 168 Hr | 168 Hr |
| Blood | 23.4 | 24.4 | 1.9 | 1.21 |
| Plasma | 23.1 | 25.4 | 1.8 | 0.7 |
| Liver | 82.0 | 74.5 | 8.6 | 15.6 |
| Gall Bladder | 71.7 | 22.4 | 2.9 | 3.8 |
| Bile | 293 | 50.6 | 7.2 | 4.4 |
| Pancreas | 11.2 | 35.1 | 2.8 | 1.3 |
| Spleen | 17.0 | 1.89 | 3.0 | 1.3 |
| Kidneys | 48.2 | 59.1 | 3.5 | 1.9 |
| Adrenals | 18.7 | 22.1 | 8.0 | 3.2 |
| Lungs | 17.6 | 21.1 | 2.0 | 1.2 |
| Heart | 19.8 | 16.5 | 2.2 | 1.2 |
| Skel. M. | 16.6 | 11.6 | 1.8 | 1.8 |
| Abdom. M. | 10.4 | 12.3 | 1.2 | 1.5 |
| Skin | 14.3 | 13.3 | 2.5 | 1.3 |
| Fat | - | - | 5.7 | 5.4 |
| Stomach* | 35.4 | 180 | 2.2 | 0.6 |
| Small Int.* | 59.2 | 45.6 | 2.6 | 0.9 |
| Cecum* | 76.7 | 55.1 | 2.9 | 0.6 |
| Large Int.* | 16.0 | 16.9 | 3.9 | 0.9 |
| Ovaries | 17.1 | - | 1.9 | 1.3 |
| Sex T.** | 23.2 | 26.9 | 1.8 | 0.9 |
| U. Bladder | 55.1 | 34.1 | 1.2 | 0.4 |
| U. Bl. Urine | - | - | 0.1 | 0.1 |
| Brain | 18.3 | 26.2 | 0.7 | 0.7 |
| Eyes | 16.5 | 13.6 | 0.0 | 0.0 |
| Bone M. | 20.1 | 24.2 | 2.3 | 1.6 |
| Aorta | - | - | 1.5 | 0.7 |
| Orbital Fat | - | - | 4.1 | - |
| Cardiac Fat | - | 19.3*** | 3.1 | 2.6 |

* Tissue plus contents.

** Uterus, vagina and fallopian tubes.

*** Brown fat.

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## TABLE 50. TISSUE DISTRIBUTION OF BCIE (AS $^{14}C$) IN MONKEYS

SINGLE ORAL DOSE OF 30 MG/KG

| | % Dose Administered | | | |
|---|---|---|---|---|
| | 111♀ | 116♀ | 290F ♀ | 75-6♀ |
| Tissue | 4 Hr | 6 Hr | 168 Hr | 168 Hr |
| Blood | 7.13 | 7.44 | 0.57 | 0.34 |
| Liver | 6.44 | 6.39 | 0.48 | 1.12 |
| Gall Bladder | 0.12 | 0.02 | <0.01 | <0.01 |
| Bile | 1.35 | 0.30 | 0.01 | 0.01 |
| Pancreas | 0.09 | 0.15 | 0.01 | 0.01 |
| Spleen | 0.04 | 0.08 | 0.01 | 0.01 |
| Kidneys | 0.63 | 0.83 | 0.03 | 0.02 |
| Adrenals | 0.02 | 0.02 | <0.01 | <0.01 |
| Lungs | 0.41 | 0.41 | 0.03 | 0.01 |
| Heart | 0.20 | 0.20 | 0.02 | 0.01 |
| Skel. M.† | 10.16 | 7.09 | 1.07 | 1.10 |
| Abdom M.†† | 3.18 | 3.75 | 0.35 | 0.44 |
| Skin††† | 0.01 | 0.01 | <0.01 | <0.01 |
| Fat†††† | - | - | 1.4 | 0.96 |
| Stomach* | 0.58 | 5.02 | 0.06 | 0.04 |
| Small Int.* | 3.43 | 1.87 | 0.13 | 0.04 |
| Cecum* | 2.81 | 3.93 | 0.07 | 0.02 |
| Large Int.* | 1.95 | 4.21 | 0.32 | 0.06 |
| Ovaries | 0.01 | - | <0.01 | <0.01 |
| Sex T.** | 0.27 | 0.63 | 0.03 | <0.01 |
| U. Bladder | 0.13 | 0.18 | 0.01 | <0.01 |
| Brain | 1.14 | 1.23 | 0.04 | 0.06 |
| Eyes | 0.13 | 0.08 | 0.00 | 0.0 |
| Bone M. | 0.02 | 0.02 | <0.01 | <0.01 |
| Aorta | - | - | <0.01 | <0.01 |
| Orbital Fat | - | - | <0.01 | - |
| Cardiac Fat | - | 0.01*** | <0.01 | <0.01 |

† Skeletal muscle, calculated as 20% body weight.
†† Abdominal muscle, calculated as 10% body weight.
††† %/g or µg/g.
†††† Fat calculated as 8% body weight.
* Tissue plus contents.
** Uterus, vagina and fallopian tubes.
*** Brown fat.

Blood samples were taken before treatment (0 blood sample) and at 1, 2, 4, 8 and 24 hours after treatment on days 1, 2 and 3. One animal (No. 98) received only 2 doses and this is indicated on the tables and graphs. On days 4 to 10 blood samples were collected at the same time on each day equivalent to the 0 sample. Hematocrits were obtained on all blood samples, and after two aliquots were prepared for LSC the remaining blood was centrifuged and one or more plasma aliquots were counted (one monkey, No. 1, was also bled on days 13, 15 and 17).

Urine samples were collected whenever the monkeys were bled if a sample was available. On day 1 urine was collected at 12 and 24 hours and thereafter at 24 hour intervals.

Feces were collected whenever available during the first 3 days, at 12 and 24 hours on day 4 and 24 hour intervals for the duration of the experiment.

Whole blood, plasma, urine and feces were analyzed for total $^{14}C$ content employing standard procedures used in this laboratory. All samples were corrected for quenching using internal standardization.

## Results - Physiological Effects

The oral treatments with BCIE produced different effects in the three monkeys. One monkey, No. 96, exhibited no reaction while the other two animals both developed evidence of local or systemic effects in the tissues around the eyes (Table 51). These ocular effects appeared to be roughly dose-related in the sensitive monkeys (1 and 98). The ocular symptoms were most prominent in Monkey 98 and, in fact, this animal was sufficiently toxic to make blood sampling a problem, and therefore BCIE was discontinued on this monkey after the second dose. After the first dose had been administered glucosuria had been verified in her 0 hr urine by Bililabstix* tests. This glucosuria persisted in all subsequent urine samples. Hyperglycemia was then verified by SMA 12/60 assay. Her general weakness, ocular changes, poor nutriture and urine flow militated against further dosing. It was also noted that the blood of this monkey at this time looked pale and abnormal in color, even though the hematocrit was not that severely affected. The ocular symptoms regressed slowly and were no longer detectable 9 or 10 days after treatment.

All three monkeys lost weight after treatment with BCIE but the weight change on day 8 for Monkey 96 was only 0.1 kg. The other two monkeys did not eat well and lost 0.4 kg. The weights of all three monkeys were the same on day 11 as on day 8.

Treatment with BCIE also had a pronounced effect on urine flow in the same monkeys which showed the ocular effects of the compound (Figure 16). Monkey 1 had depressed urine flow for 17 days. Although she was gavaged

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*Bililabstix - Reagent strips which test for pH, protein, glucose, ketones, bilirubin and blood in urine. Supplied by Ames Company, Division of Miles Laboratories, Inc., Elkhart, Indiana 46514.

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with 75 ml of $H_2O$ on days 2, 3 and 15, no improvement in urine flow was observed except on day 16, when 118 ml of urine was excreted. Monkey 98 also showed reduction in urine flow which, however, was corrected as soon as dosing was discontinued. Details of the urine excretion of BCIE will be considered later.

Blood levels of BCIE in the 3 monkeys are detailed in Figure 17. Peak concentrations ranging from 17 to 34 μg/ml occurred 2 hours after treatment, after which the levels dropped rapidly for the next 6 hours and more slowly between the 8 and 24 hour period. One observes increasing residual radioactivity in the blood with repeated doses (Table 52, Figure 17). If all three monkeys had received 3 consecutive doses, one could conclude that the average trough level at 24 hours after the third dose would have appreciated from 50 to 100%.

On the other hand, there is no clear cut indication of rising peak concentrations with repeated doses, indicating a reasonable maintenance of the early "α" phase of the dieaway curve; however, even the limited data obtained in this study suggests that the slope of the dieaway curves is flatter on day 3 than previously.

Plasma levels of BCIE were regularly higher and follow essentially the same patterns observed with whole blood (Table 53 and Figure 18). The evidence of toxicity in Monkey 98 on the morning of day 3 correlates with her high residual $^{14}$C activity from the previous dose as well as her high peak reading (41 μg/ml) observed after administration of the second dose. After dosing was discontinued the levels in plasma fell, but even at 7 days all levels were still above 5 μg/ml (as $^{14}$C).

The data on urinary excretion of BCIE expressed as $^{14}$C are presented in two ways. In Table 54 and Figure 19 the data on urinary radioactivity in mg of BCIE equivalents indicate the variability in response observed. The low recovery in Monkey 1 on day 2 is, of course, due to the animal's having vomited an unknown, (ca 50%?) but obviously significant, portion of its second dose of BCIE. The omission of the third dose for Monkey 98 prevents comparison with the "3 dose" monkeys. As discussed previously, this monkey had poor urine flow. When dosing ceased, urine flow improved promptly but only small additional amounts of $^{14}$C were recovered in the urine.

If we consider the excretion of drug in the urine in % dose one can generate the upper and lower limit values (single dose vs. additive dose calculations, Table 55) but the absolute values for % dose would depend on one's knowing how much residual BCIE remained in the monkey at the end of the first day, the second day, etc. Since much of the BCIE is lost by exhalation we cannot establish this residual from data on urine and feces recovery. Nevertheless, the data in Figures 20, 21 and 22 support the conclusion that although only 30-45% of the dose is accounted for in the urine (and feces) the rest of the radioactivity must have been lost by another route, exhalation.

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In the case of Monkey 1, (Figure 20), the data are rather vague (because of the vomiting of the second dose). Nevertheless, one is impressed by the small amount of $^{14}C$ recovered during the 3rd day, less than 15%, based on single dose calculations. Thus, this may indicate that excretion of BCIE in the urine is dependent on urine flow. The data on Monkey 1 which had very poor urine flow on days 1, 2 and 3 and thereafter suggests that urine flow is critical.

In the case of Monkey 96 (Figure 21) one observes a fairly consistent urinary excretion pattern with somewhat lower recovery figures on the second day, possibly related to the relatively lower figures on this day for BCIE in plasma (Table 53, Figure 18).

Monkey 98 whose data are shown in Figure 22 has very consistent values for urinary excretion of $^{14}C$, roughly 26% of dose based on single dose calculation; however, consideration of the plasma concentration data in Table 53 and Figure 18 indicates that this monkey had relatively high residual $^{14}C$ 24 hours after the first dose of BCIE and the highest levels of $^{14}C$ of any monkey after the second dose. Concomitantly, a great reduction in urine flow was observed. These findings probably account for the high degree of ocular changes observed and the overall toxicity observed which necessitated discontinuation of dosing.

Turning to the figures for fecal excretion of $^{14}C$ from BCIE (Table 56) one notes that excretion via this route never accounts for more than 4% of the dose (based on single dose of 30 mg/kg). It is obvious that at least a small fraction of the dose in every case is still present as residual $^{14}C$ 24 hours after the BCIE was administered. Therefore, one can be certain that no more than about 3% of the $^{14}C$ from BCIE is excreted in the feces even under the extreme conditions of low urine flow observed in Monkeys 1 and 98. One can conclude, in fact, that fecal excretion is a very minor pathway with BCIE and that whatever $^{14}C$ is excreted in the bile is either relatively little or is well reabsorbed via the enterohepatic circulation.

## Discussion

This experiment points out some very interesting findings. One, that some monkeys are highly susceptible to the toxic side effects of BCIE on the ocular and renal systems and possibly on other organ systems not studied. Some monkeys (like 96), however, exhibit neither the ocular nor the renal effects, and show little effect on appetite or weight. Whether or not there is any direct connection between these toxic manifestations is not known.

It is obvious that only a small fraction of the $^{14}C$ from BCIE is bound to tissues or fat, either at 4-6 hours or 168 hours after dosing (see data on tissue distribution in Quarterly Report 76-4); therefore, most of the compound (>60% of the dose) must be exhaled. However, it is difficult to understand how the pulmonary clearance of the compound can account for the eye irritation, since in the previous animals which received 30 mg/kg and in 96 in this series the same proportion of the dose was apparently exhaled, yet only 1 and 98 exhibited both the severe eye toxicity and effects on urine flow. We maintain careful records on menstrual cycles of our rhesus

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stock. The menstrual cycle of Rhesus 1 may have been affected by BCIE. Her 5-day cycle ended 6 days before dosing was instituted and menses recurred on the fifth day of this experiment - some 14 days before it was expected. No other menstrual deviations were observed.

No explanation is available from these studies to account for the large differences in the way different monkeys respond to this compound, although one monkey exhibited hyperglycemia and glucosuria before and after the administration of BCIE. How this affected the response to BCIE is not known at this time.

## Conclusions

1. A single oral dose of BCIE (30 mg/kg) produced marked toxic effects on the eyes of 2 of 3 monkeys studied and these changes were intensified with repeated doses.

2. The same monkeys exhibiting ocular toxicity also showed very great reductions in urine flow. These were readily reversed in one monkey following cessation of treatment but persisted in a second for 12 days.

3. A third monkey exhibited no apparent side effects from 3 oral 30 mg/kg doses of BCIE, but residual blood and plasma levels of $^{14}C$ rose with each dose, suggesting the possibility of subacute toxic effects if the dosing had been continued as planned.

4. As noted in previous studies, very little $^{14}C$ from BCIE is excreted in the feces indicating rapid and complete absorption of oral doses and apparent facile absorption of any $^{14}C$ excreted via the bile.

5. One monkey may have had an early menstrual cycle as a result of BCIE.

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## TABLE 51. OCULAR EFFECTS OF BCIE

| Time Post Treatment Day | Hr | 1 ♀ | 96 ♀ | 98 ♀ |
|---|---|---|---|---|
| 0 | | 0 | 0 | 0 |
| 1 | 4 | ++ | 0 | ++ |
| | 12 | + | 0 | + |
| | 24 | ± | 0 | ± |
| 2 | 4 | +++ | 0 | ++++ |
| | 12 | ++ | 0 | +++ |
| | 24 | ++ | 0 | +++ |
| 3 | | +++ | 0 | +++ (no treatment) |
| 4 | | +++ | 0 | ++ |
| 5 | | ++ | 0 | ++ |
| 6 | | + | 0 | ++ |
| 7 | | ± | 0 | + |
| 8 | | ± (0.4 kg wt loss) | 0 (0.1 kg wt loss) | + (0.4 kg wt loss) |
| 9 | | ± | 0 | ± |
| 10 | | 0 | 0 | ± |
| 11 | | 0 | 0 | ± |
| 12 | | 0 | 0 | ± |

± Minimal changes.

0 No visible effects.

± Regressing symptoms.

+ Edema, discoloration.

++ Prominent edema, sl. hemorrhage (?)

+++ Marked swelling, edema, prominent subdural hemorrhage (?)

++++ Very intense effects, eyes essentially swollen shut.

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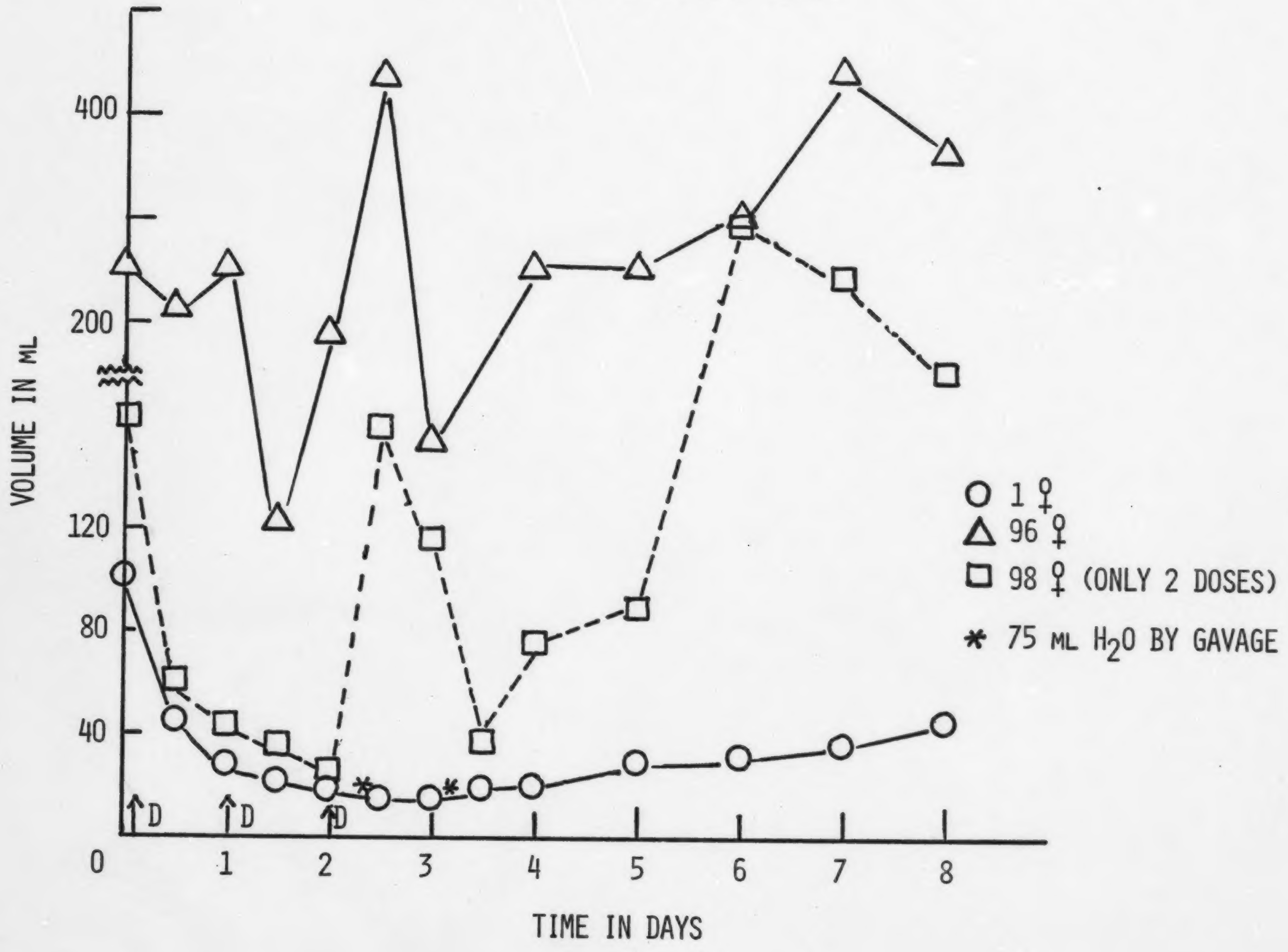
FIGURE 16
URINE VOLUMES IN RHESUS MONKEYS BEFORE AND AFTER
3 ORAL DOSES OF BCIE 30 MG/KG



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## TABLE 52. BLOOD CONCENTRATIONS OF BCIE (AS $^{14}C$) IN MONKEYS

## THREE CONSECUTIVE DAILY ORAL DOSES OF 30 MG/KG

| Time Post Treatment | | 1 ♀ | | 96 ♀ | | 98 ♀* | |
|---|---|---|---|---|---|---|---|
| Day | Hr | % Hct | µg/g | % Hct | µg/g | % Hct | µg/g |
| 1 | 0 | 48.5 | 0 | - | 0 | 47 | 0 |
| | 1 | 48.5 | 16.5 | 46 | 20.8 | 39 | 23.1 |
| | 2 | 48 | 17.2 | 46 | 27.8 | 44 | 29.9 |
| | 4 | 46 | 17 | 44 | 20.4 | 46.5 | 21.0 |
| | 8 | 50.5 | 17.1 | 46 | 15.2 | 46.0 | 14.3 |
| | 24 | 46 | 8.9 | 45 | 5.0 | 45.5 | 8.2 |
| 2 | 1 | 46.5 | 16.5 | 44 | 23.0 | 44.5 | 25.2 |
| | 2 | 41 | 13.7 | 43 | 22.6 | 43 | 34 |
| | 4 | 44 | 12.2 | 43 | 18.7 | 42 | 26.7 |
| | 8 | 42.5 | 10.4 | 41 | 12.4 | 40 | 16.1 |
| | 24 | 40 | 7.8 | 41 | 8.3 | 38 | 10.5 |
| 3 | 1 | 40 | 30.7 | 40.5 | 28.6 | - | - |
| | 2 | 39 | 31.7 | 41.5 | 30.9 | - | - |
| | 4 | 38 | 27.6 | 39.5 | 22.0 | - | - |
| | 8 | 39 | 23.2 | 39.5 | 14.3 | - | - |
| | 24 | 36.5 | 14.9 | 38.5 | 10.0 | 36.5 | 8.3 |
| 4 | | 35.5 | 11.6 | 38 | 8.4 | 36 | 7.4 |
| 5 | | 38.0 | 10.6 | 37 | 8.6 | 37 | 9.0 |
| 6 | | 38.5 | 8.8 | 38 | 7.3 | 37 | 6.6 |
| 7 | | 38 | 8.9 | 39.5 | 7.5 | 40 | 6.5 |
| 8 | | 43 | 8.0 | 42.5 | 6.8 | 41 | 6.1 |
| 9 | | 39 | 7.3 | 40.5 | 6.6 | 38.5 | 6.0 |
| 10 | | 44 | 7.0 | 41.5 | 5.9 | 42 | 5.7 |
| 13 | | 48 | 5.7 | | | | |
| 15 | | 43.5 | 5.5 | | | | |
| 17 | | - | 4.9 | | | | |

*98 ♀ received only 2 doses.

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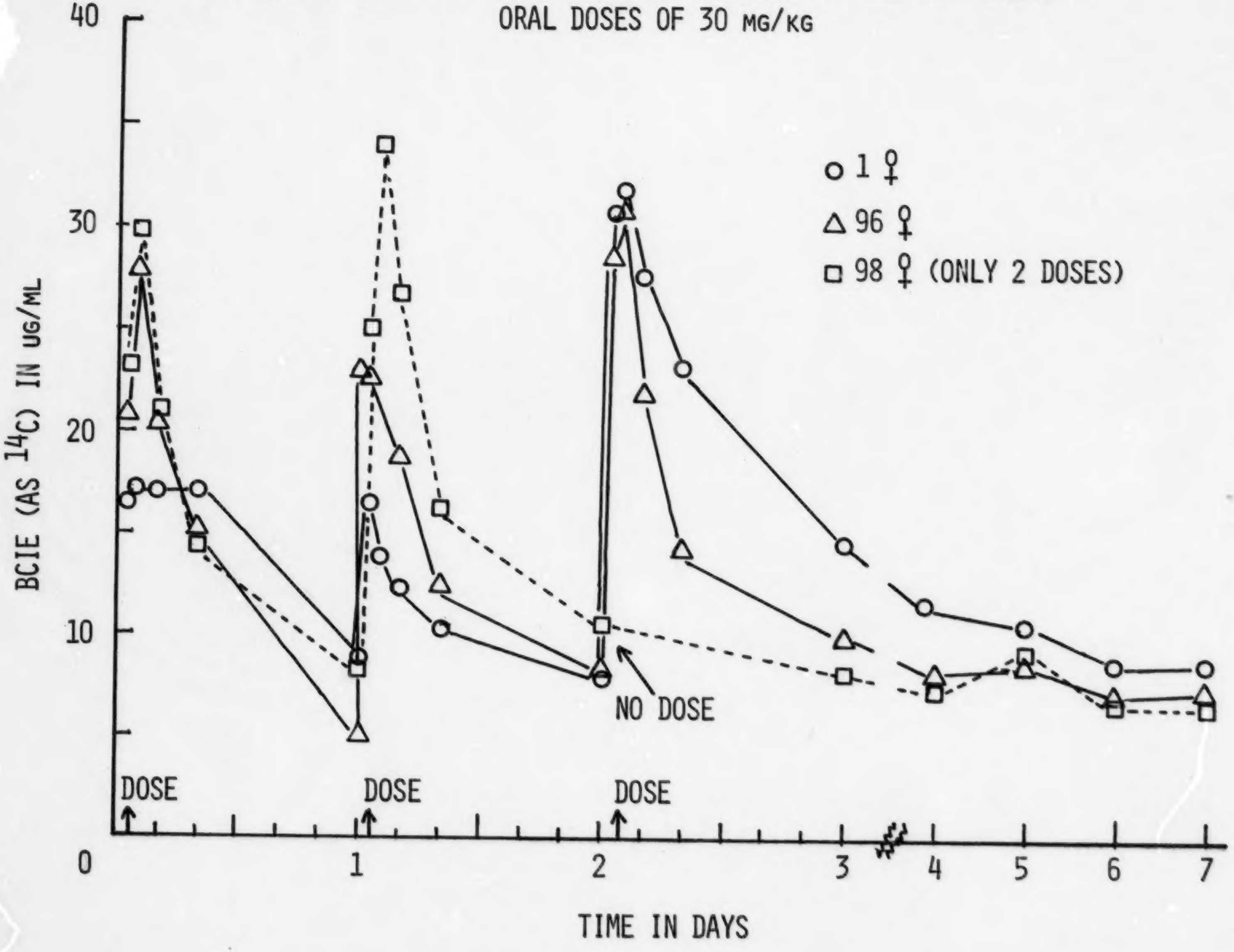
FIGURE 17
CONCENTRATION OF BCIE (AS $^{14}C$) IN BLOOD OF RHESUS MONKEYS
ORAL DOSES OF 30 MG/KG



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## TABLE 53. PLASMA CONCENTRATIONS OF BCIE (AS $^{14}C$) IN MONKEYS

THREE CONSECUTIVE DAILY ORAL DOSES OF 30 MG/KG

| Time Post Treatment<br>Day | <br>Hr | 1 ♀<br>μg/g | 96 ♀<br>μg/g | 98 ♀*<br>μg/g |
|---|---|---|---|---|
| | 0 | 0 | 0 | 0 |
| 1 | 1 | 19.1 | 25.5 | 28.4 |
| | 2 | 19.0 | 32.0 | 32.1 |
| | 4 | 15.7 | 21.1 | 21.9 |
| | 8 | 20.3 | 18.2 | 16.5 |
| | 24 | 13.2 | 6.6 | 12.4 |
| 2 | 1 | 24.1 | 28.2 | 31.2 |
| | 2 | 19.3 | 26.5 | 40.7 |
| | 4 | 18.1 | 21.9 | 32.0 |
| | 8 | 16.7 | 14.7 | - |
| | 24 | 10.6 | 10.4 | 16.0 |
| 3 | 1 | 37.7 | 36.1 | - |
| | 2 | 37.7 | 37.5 | - |
| | 4 | 31.4 | 24.9 | - |
| | 8 | 28.5 | 19.6 | - |
| | 24 | 20.0 | 13.4 | 10.2 |
| 4 | | 14.5 | 10.5 | 8.8 |
| 5 | | 12.0 | 9.8 | 10.1 |
| 6 | | 9.5 | 8.4 | 7.1 |
| 7 | | 7.5 | 7.3 | 6.0 |
| 8 | | 7.1 | 6.4 | 5.1 |
| 9 | | 6.4 | 6.0 | 4.8 |
| 10 | | 5.9 | 5.4 | 4.8 |
| 13 | | 4.5 | | |
| 15 | | 3.8 | | |
| 17 | | 3.3 | | |

* 98 ♀ received only 2 doses.

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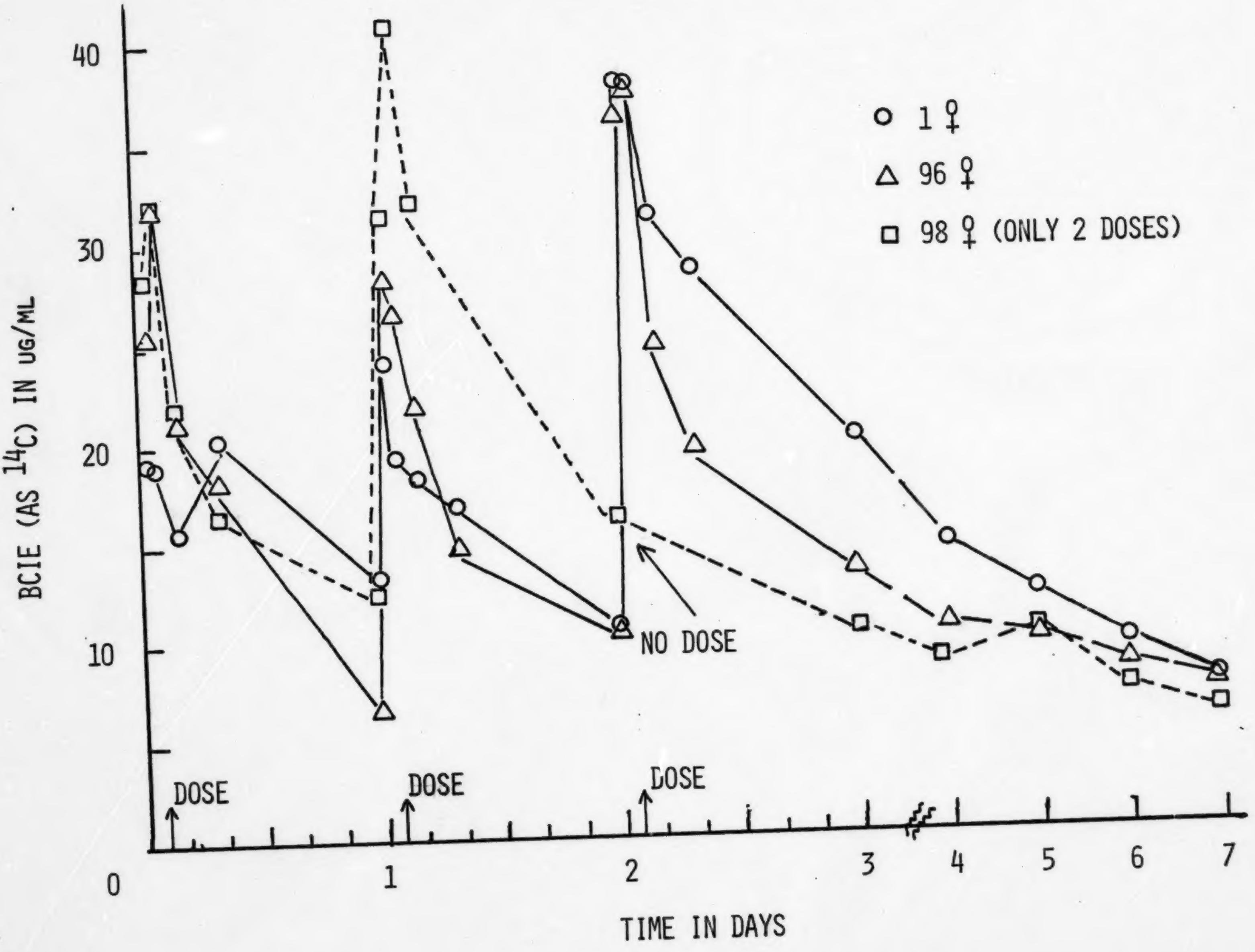
FIGURE 18
CONCENTRATION OF BCIE (AS $^{14}C$) IN PLASMA IN RHESUS MONKEYS
3 ORAL DOSES OF 30 MG/KG



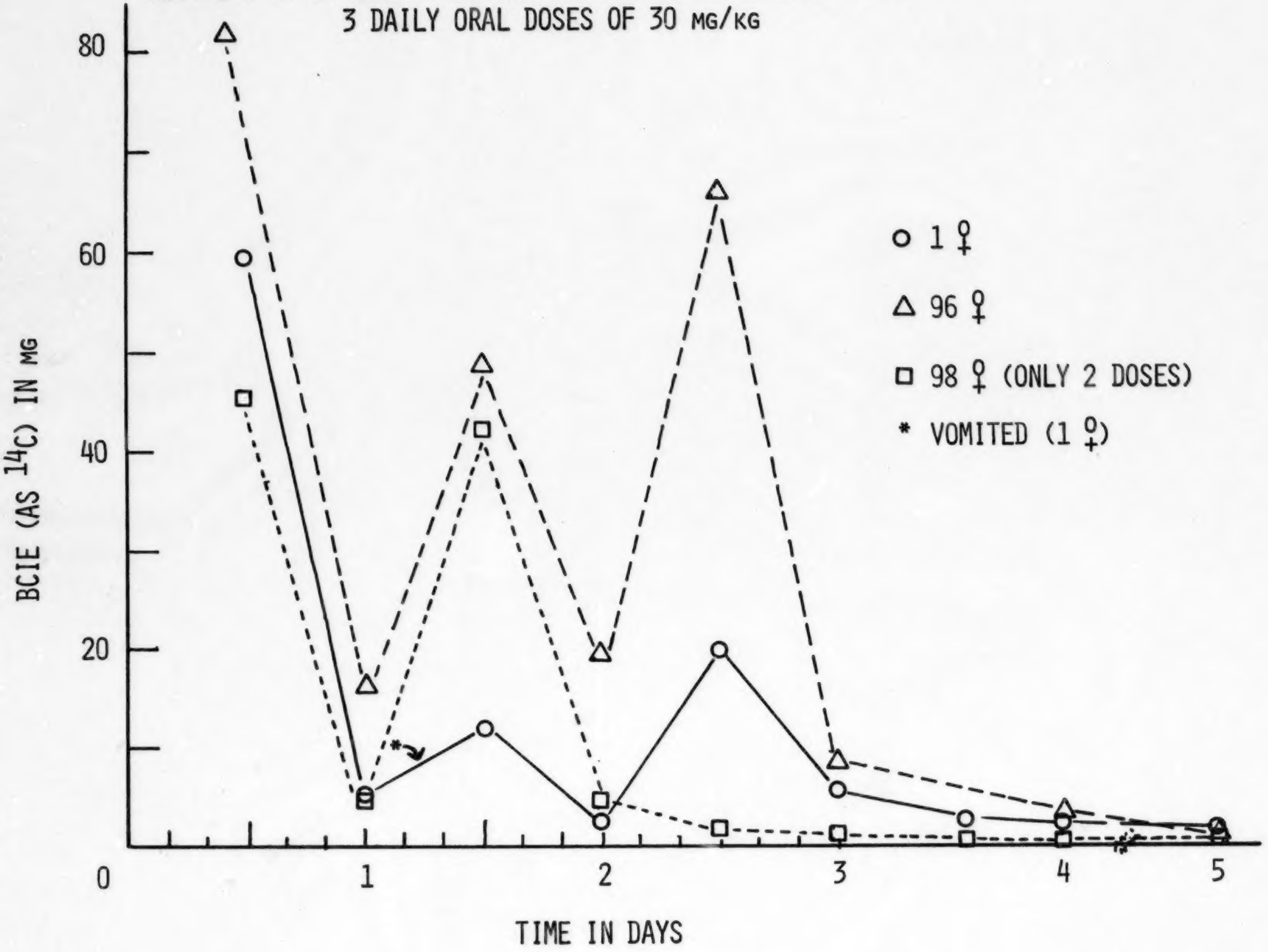
## TABLE 54. RECOVERY OF BCIE IN MONKEY URINE

30 MG/KG ONCE DAILY FOR 3 DAYS

| Time Post Treatment | | Total mg as $^{14}C$ | | |
|---|---|---|---|---|
| Day | Hr | 1 ♀ | 96 ♀ | 98 ♀* |
| 1 | 12 | 59.69 | 82.00 | 45.56 |
| | 24 | 5.19 | 16.49 | 4.35 |
| 2 | 12 | 12.00 | 49.04 | 42.12 |
| | 24 | 2.45 | 19.85 | 4.71 |
| 3 | 12 | 20.02 | 66.26 | 1.72 |
| | 24 | 5.78 | 8.58 | 1.23 |
| 4 | 12 | 2.65 | NS | 0.39 |
| | 24 | 2.23 | 3.49 | 0.38 |
| 5 | 24 | 1.84 | 1.28 | 0.57 |
| 6 | 24 | 1.10 | 0.84 | 0.65 |
| 7 | 24 | 0.87 | 0.54 | 0.37 |
| 8 | 24 | 0.60 | 0.48 | 0.30 |
| 9 | 24 | 0.44 | 0.35 | 0.27 |
| 10 | 24 | 0.29 | 0.32 | 0.21 |

* 98 ♀ received only 2 doses.

FIGURE 19
RECOVERY OF URINARY BCIE (AS $^{14}C$) IN MG IN RHESUS MONKEYS
3 DAILY ORAL DOSES OF 30 MG/KG



## TABLE 55. CUMULATIVE RECOVERY OF BCIE (AS $^{14}C$) IN MONKEY URINE BASED ON SINGLE DOSE (30 MG/KG) OR ADDITIVE DOSE (30, 60, 90 MG/KG)

3 CONSECUTIVE ORAL DAILY DOSES OF 30 MG/KG

| Time Post Treatment | | % Dose | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 ♀ | | 96 ♀ | | 98 ♀* | |
| Day | Hr | Single Dose | Additive Dose | Single Dose | Additive Dose | Single Dose | Additive Dose |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | 1 | NS | NS | 4.02 | 4.02 | 2.10 | 2.10 |
| | 2 | 6.86 | 6.86 | 10.85 | 10.85 | 8.71 | 8.71 |
| | 4 | 10.96 | 10.96 | 26.28 | 26.28 | 12.37 | 12.37 |
| | 8 | 22.19 | 22.19 | 36.39 | 36.39 | 20.17 | 20.17 |
| | 12 | 28.70 | 28.70 | 36.58 | 36.58 | 23.72 | 23.72 |
| | 24 | 31.19 | 31.19 | 43.93 | 43.93 | 25.98 | 25.98 |
| 2 | 1 | NS | NS | NS | NS | 1.57 | 0.79 |
| | 2 | NS | NS | 0.12 | 0.06 | 5.81 | 2.91 |
| | 4 | 4.93 | 2.46 | 12.15 | 6.07 | 10.57 | 5.29 |
| | 8 | 5.09 | 2.55 | 12.38 | 6.18 | 20.75 | 10.38 |
| | 12 | 5.76 | 2.89 | 21.90 | 10.94 | 21.93 | 10.97 |
| | 24 | 6.93 | 3.48 | 30.75 | 15.37 | 24.38 | 12.20 |
| 3 | 1 | NS | NS | 4.20 | 1.40 | - | - |
| | 2 | NS | NS | 12.38 | 4.17 | - | - |
| | 4 | 3.73 | 1.25 | 23.08 | 7.74 | - | - |
| | 8 | 9.63 | 3.22 | 29.35 | 9.83 | - | - |
| | 12 | NS | NS | 29.44 | 9.86 | 25.27 | 12.64 |
| | 24 | 12.41 | 4.15 | 33.13 | 11.09 | 25.90 | 12.97 |
| 4 | 12 | 13.69 | 4.57 | - | - | 26.11 | 13.07 |
| | 24 | 14.75 | 4.92 | 34.68 | 11.61 | 26.31 | 13.17 |
| 5 | 24 | 15.63 | 5.22 | 35.26 | 11.80 | 26.60 | 13.31 |
| 6 | 24 | 16.16 | 5.40 | 35.63 | 11.92 | 26.93 | 13.47 |
| 7 | 24 | 16.59 | 5.54 | 35.87 | 12.00 | 27.12 | 13.57 |
| 8 | 24 | 16.89 | 5.64 | 36.08 | 12.07 | 27.27 | 13.64 |
| 9 | 24 | 17.10 | 5.71 | 36.23 | 12.12 | 27.41 | 13.71 |
| 10 | 24 | 17.25 | 5.76 | 36.37 | 12.17 | 27.52 | 13.76 |

*98 received only two doses.

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FIGURE 20
CUMULATIVE URINARY EXCRETION OF BCIE (AS $^{14}C$) IN 1 ♀
3 ORAL DOSES OF 30 MG/KG

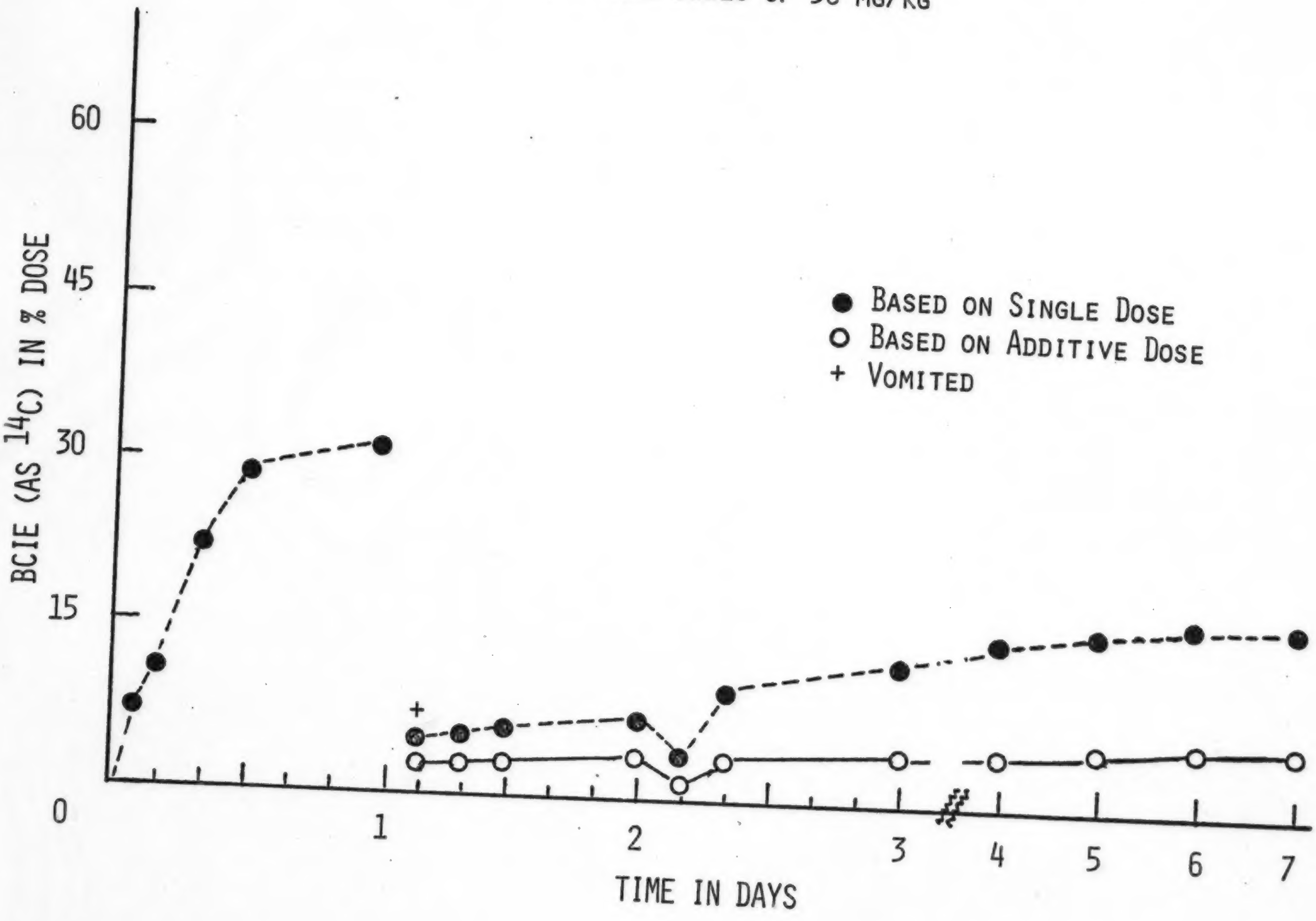


FIGURE 21
CUMULATIVE URINARY EXCRETION OF BCIE (AS $^{14}C$) IN 96♀
3 ORAL DOSES OF 30 MG/KG

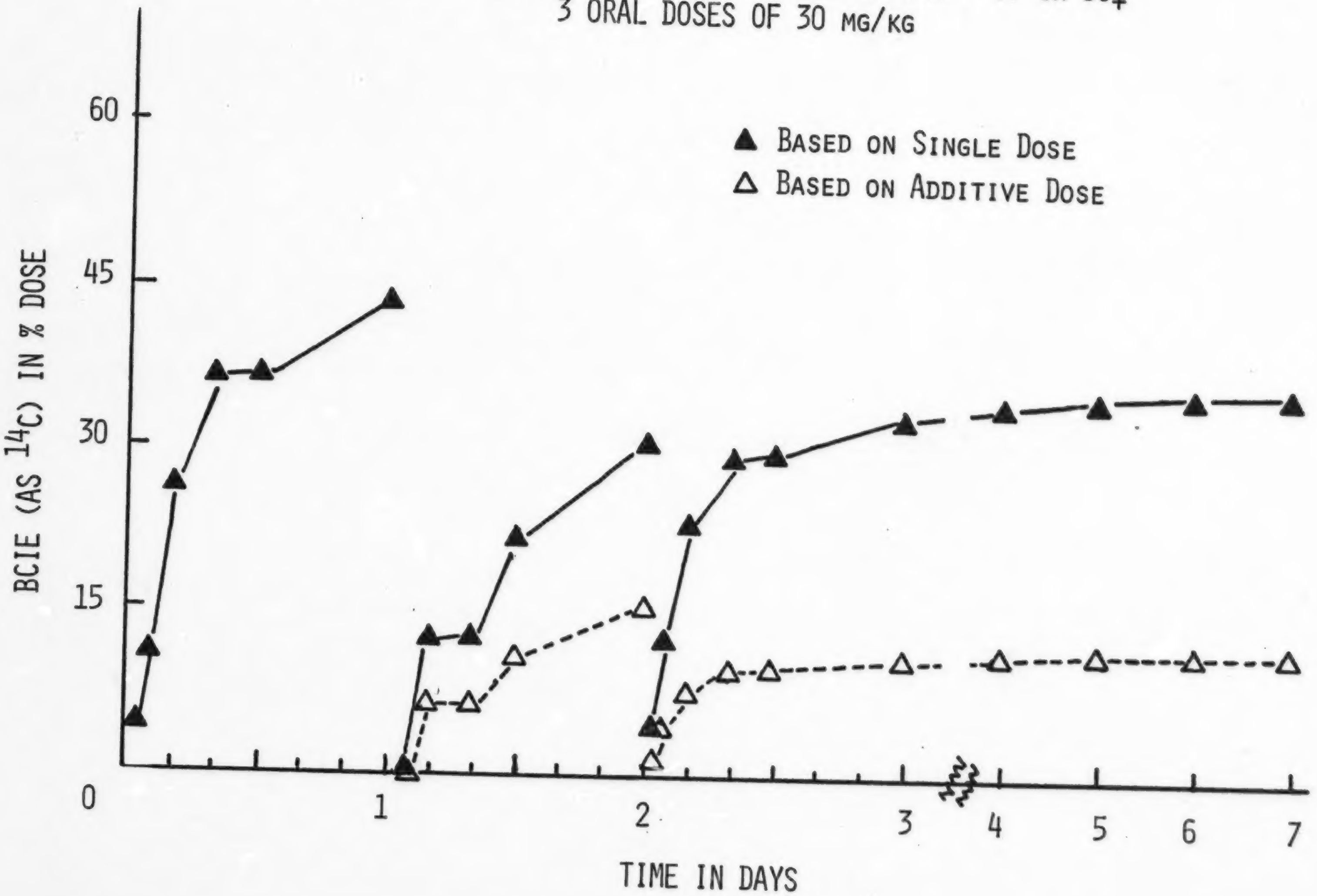
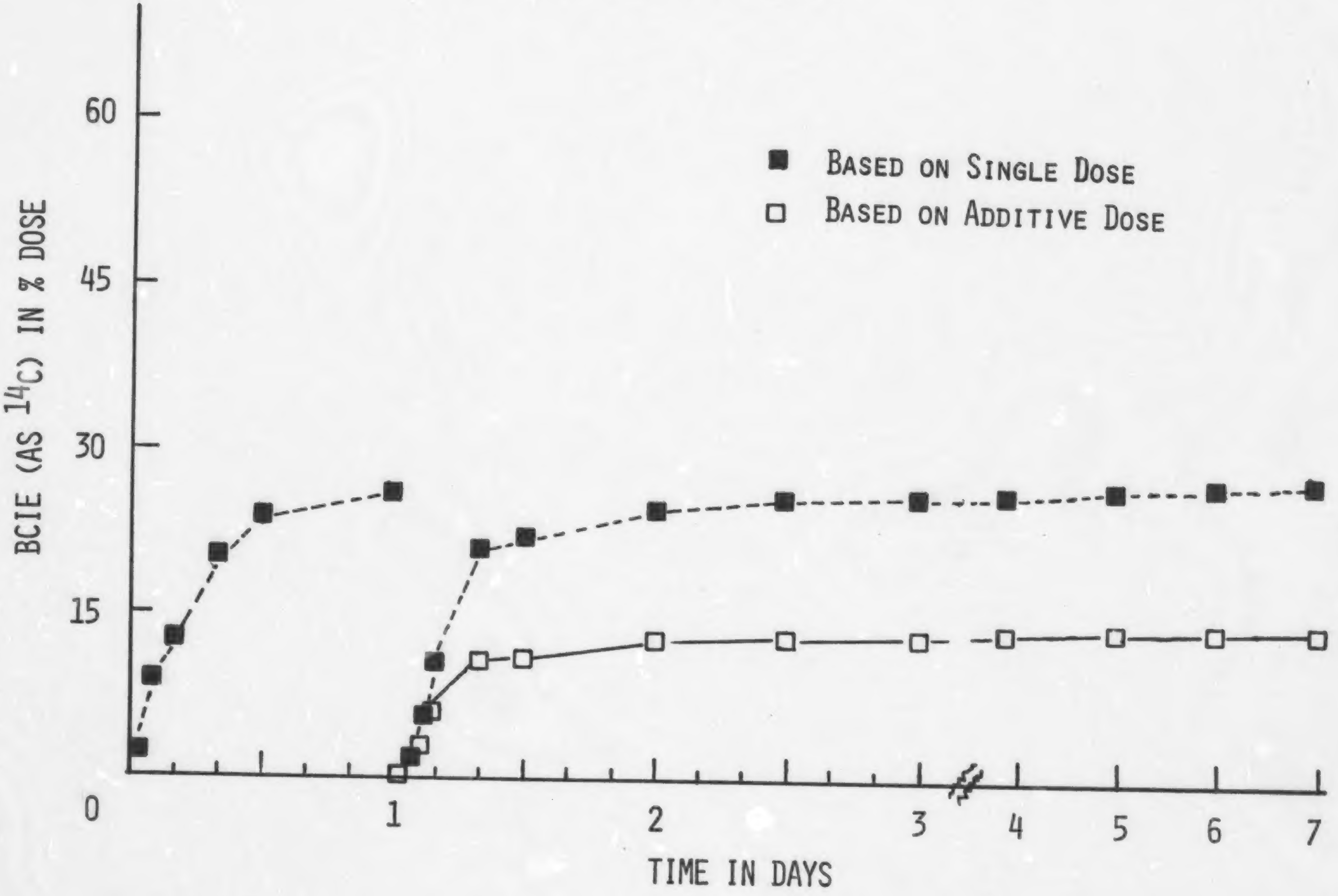


FIGURE 22
CUMULATIVE URINARY EXCRETION OF BCIE (AS $^{14}C$) IN 98♀
2 ORAL DOSES OF 30 MG/KG



## TABLE 56. CUMULATIVE RECOVERY OF BCIE (AS $^{14}C$) IN MONKEY FECES BASED ON SINGLE DOSE (30 MG/KG) OR ADDITIVE DOSE (30, 60, 90 MG/KG)

3 CONSECUTIVE ORAL DAILY DOSES OF 30 MG/KG

| Time Post Treatment | | % Dose | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1 ♀ | | 96 ♀ | | 98 ♀* | |
| Day | Hr | Single Dose | Additive Dose | Single Dose | Additive Dose | Single Dose | Additive Dose |
| 1 | 12 | 0.45 | 0.45 | 0.90 | 0.90 | 0.86 | 0.86 |
| | 24 | - | - | 1.25 | 1.25 | - | - |
| 2 | 12 | 0.08 | 0.04 | 0.92 | 0.46 | 0.71 | 0.36 |
| | 24 | 0.17 | 0.09 | 2.56 | 1.28 | 2.31 | 1.16 |
| 3 | 12 | 0.77 | 0.26 | 0.83 | 0.28 | - | - |
| | 24 | 1.80 | 0.60 | 1.16 | 0.39 | 3.32 | 1.66 |
| 4 | 12 | - | - | - | - | 3.41 | 1.70 |
| | 24 | 2.13 | 0.71 | 2.52 | 0.84 | 3.65 | 1.82 |
| 5 | | 2.51 | 0.84 | 2.81 | 0.94 | 3.71 | 1.85 |

* 98 ♀ received only 2 doses.

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## SECTION 8

## PHARMACOKINETIC STUDY OF BIS(2-CHLOROETHYL)ETHER IN TWO RHESUS MONKEYS

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### Introduction

Bis(2-chloroethyl)ether (BCEE) is one of a group of haloethers which are widespread environmental contaminants and have been detected in drinking water. BCEE is used as a dewaxing agent, solvent, cleaning agent and penetrant (EPA Criterion Document, July 9, 1979). Lingg *et al*. (1978) reported observations on the metabolic fate of BCEE in rats. Smith and Lingg summarized the current information of metabolism of β-haloethers in rats and monkeys at a recent symposium(22). BCEE has been studied pharmacokinetically in two rhesus monkeys and these data comprise this report.

### Materials and Methods

The treatment solution for oral administration was prepared as follows: 4.0 ml of ethanol was mixed with 4.3 ml of Emulphor®. To this was added 0.39 g of cold BCEE, slightly more than 0.02 ml of $^{14}$C-BCEE (0.56 μCi/ml), and 31.7 g of deionized distilled water. Liquid scintillation assay of this solution established the specific activity to be 0.49 μCi/ml (1,090,600 dpm) and 106.27 dpm/μg of BCEE.

The two female rhesus monkeys weighed 8.9 and 9.75 kg, respectively. The monkeys were dosed by oral gavage with a single 10 mg/kg dose in the A.M. Each was housed in a metabolism cage maintained within an exposure chamber which exhausted all the exhaled compound. Each monkey wore a collar with a chain that hooked to the cage door. The monkeys were fed 10-15 pellets of Purina chow per day and provided water *ad libitum*. Urine samples were collected (if available) at 6, 12, 24, 36, 48, 72 and 96 hours after treatment. Collection flasks were iced over the entire collection time. Feces samples were collected at 24, 48, 72 and 96 hours.

Both urine and feces samples were processed by standard procedures of this laboratory for liquid scintillation counting. In addition, all urines were tested with Bililabstix at the time of measuring samples and taking aliquots for LSC counting.

### Results

Data on cumulative excretion of bis(2-chloroethyl)ether in urine and feces of the two monkeys is presented in Table 57. The 6-hour urine sample on Rhesus 1 contained 17% of the dose. At 12 hours this monkey had excreted ca 30% of the dose in urine and at 24 hours this increased to 43%. It is interesting that Rhesus 96 also excreted 43% of the dose in urine by the time lapse of 24 hours, but this monkey voided only 26.5 ml of urine before the 24 hour collection time (see urine volumes for both monkeys listed in Table 58. By 72 hours we recovered 53% of the dose in urine for Rhesus 1 and 63% of the dose in urine for Rhesus 96. We did not collect 96-hour

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urine on Rhesus 1 since she was in menses and was returned to her stock cage after 72 hours. However, the 72-96 hour urine on Rhesus 96 contributed very little to recovery of $^{14}$C-BCEE or metabolites.

Examination of recovery data on feces in the two monkeys shows that very little of the compound was recovered in feces. Total recovery in samples through 96 hours was 1.10 and 1.63% of the dose for 1 and 96, respectively. (Table 58 shows the weight of feces samples collected at each 24-hour interval for each monkey.)

## Physiological Observations on Monkeys

Rhesus 1 began menstruation at 24 hours after dosing. Also within 24 hours hemorrhagic darkening occurred below her eyes. Ketones were present in her urine at 12, 24, 36, 48 and 72 hours. She was returned to her stock cage after 72 hours and urine collection was discontinued. An SMA 12/60 assay (Table 59) was performed on a 72-hour blood sample. The hematocrit was 41.5% The monkey's plasma LDH was elevated slightly (LDH = 368 vs. normal level of 271 mU/ml ± 24.6 S.E.). Phosphorus was also slightly elevated (5.2 vs. normal level of 3.6 mgms % ± 0.21 S.E.). This monkey ate less food and excreted much smaller urine samples than Rhesus 96.

Rhesus 96 ate very well and excreted larger volumes of urine than Rhesus 1. In this monkey 63.5% of the dose was recovered in urine and 1.6% of the dose in feces in 96 hours.

## Conclusions

BCEE was administered to 2 female rhesus monkeys in single oral doses of 10 mg/kg. From 54 to 65% of the dose as $^{14}$C was excreted in the urine and 1-1.6% in the feces. One of the monkeys showed evidence of infraorbital irritation, a finding also observed when larger (30 mg/kg) doses of Bis(2-chloroisopropyl)ether (BCIE) were administered. With $^{14}$C-BCIE recovery in urine varied from 24 to 30% and 1-1.5% was recovered in the feces. From these data we conclude that BCEE is probably more toxic for subhuman primates than BCIE and both compounds appear to be quite irritating to the eyes.

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## TABLE 57. CUMULATIVE EXCRETION OF BCEE (AS $^{14}C$) IN MONKEYS

SINGLE ORAL DOSE OF 10 MG/KG

| | Percent Dose | | | | | |
|---|---|---|---|---|---|---|
| | 1 ♀ | | | 96 ♀ | | |
| Time Post Treatment (Hours) | Urine | Feces | U & F | Urine | Feces | U & F |
| 6 | 16.66 | - | 16.66 | - | - | - |
| 12 | 29.85 | - | 29.85 | 0.003 | - | 0.003 |
| 24 | 43.43 | 0.22 | 43.65 | 43.23 | 0.04 | 43.27 |
| 36 | 48.60 | - | 48.88 | 43.50 | - | 43.54 |
| 48 | 51.03 | 0.64 | 51.67 | 61.05 | 0.09 | 61.14 |
| 72 | 53.30 | 0.76 | 54.06 | 62.96 | 0.65 | 63.61 |
| 96 | * | 1.10 | 54.40 | 63.45 | 1.63 | 65.08 |

*Monkey returned to stock cage.

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## TABLE 58. URINE VOLUMES AND FECES WEIGHTS OF $^{14}$C BCEE-TREATED MONKEYS

SINGLE ORAL DOSE 10 MG/KG

| | 1 ♀ | | 96 ♀ | |
|---|---|---|---|---|
| Time Post Treatment (Hours) | Urine Volume (ml) | Feces Weight g | Urine Volume (ml) | Feces Weight g |
| 6 | 106.5 | - | - | - |
| 12 | 14.6 | - | 26.5 | - |
| 24 | 31.2 | 2.34 | 400 | 0.47 |
| 36 | 24.0 | - | 16 | - |
| 48 | 26.0 | 16.78 | 320 | 1.05 |
| 72 | 73.0 | 1.63 | 375 | 13.68 |
| 96 | * | 12.37 | 561 | 55.06 |

*Monkey returned to stock cage.

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TABLE 59. SMA 12/60 ASSAY ON RHESUS 1♀ AT 72 HRS. FOLLOWING SINGLE ORAL 10 MG/KG/DOSE OF BIS (2-CHLOROETHYL)ETHER

| Assay | Value | Normal Mean ± SE** |
|---|---|---|
| Sugar | 95 mgm% | 89 ± 3.5 |
| Uric acid | 0.3 mgm% | 0.61 ± 0.05 |
| Cholesterol | 135 mgm% | 170.2 ± 6.0 |
| SGOT | 36 mU/ml | 30.4 ± 2.2 |
| LDH | 368 mU/ml* | 271.2 ± 24.6 |
| P | 5.2 mgm%* | 3.56 ± 0.21 |
| Alk T'ase | 78 mU/ml | 166.3 ± 17.5 |
| Ca | 7.9 mgm% | 9.78 ± 0.16 |
| Bilirubin (1 minute) | 0.0 mgm% | 0.12 ± 0.01 |
| Bilirubin (total) | 0.2 mgm% | 0.26 ± 0.02 |
| Protein (total) | 6.6 mgm% | 8.01 ± 0.13 |
| Albumin | 2.2 mgm% | 3.77 ± 0.11 |

*Deviations from normal values.

**Means established on 20 rhesus stock females of closely matched weights.

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## APPENDIX

# METABOLISM OF β-HALOETHERS IN RATS AND MONKEYS

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Cincinnati, Ohio 45268

Bis(2-chloroethyl)ether (BCEE) and bis(2-chloroisopropyl)ether (BCIE) have been identified as contaminants in some drinking water supplies. These compounds labeled with $^{14}C$, have been administered orally to rats and monkeys. As reported by Lingg et al. (1978) most of the $^{14}C$ following an oral dose of labeled BCEE in rats appeared in the urine. Some 10% was recovered in the expired air as $^{14}CO_2$ and almost none was exhaled unchanged. Using a purging technique no volatile metabolites were detected. The urine was separated by standard techniques into acid, neutral and basic fractions. The acid fraction, containing essentially all the $^{14}C$ activity, was derivatized with diazomethane and a trimethylsilylating agent (BSTFA) and analyzed by GC-MS. Two metabolites, thiodiglycolic acid and 2-chloroethyl-β-D-glucosiduronic acid, were identified in this acid fraction.

In the case of BCIE the rat excretes $^{14}C$-containing metabolites primarily in the urine and very little BCIE is excreted unchanged. Using the same purging technique, three volatile urinary metabolites were detected. These consisted of propylene oxide, 1-chloro-2-propanol and β-chloroisopropyl isopropyl ether. No sex-related differences in the rodent pattern of volatile metabolites was detected. Following isolation of the urinary acid fraction and appropriate derivatization, two metabolites were identified by GC-MS. These consisted of 2(1-methyl-2-chloroethoxy) propionic acid and the glucuronide of 1-chloro-2-propanol.

In the monkey, metabolism of labeled BCIE leads to the same 5 volatile and non-volatile urinary metabolites as were identified in rodent urine. However, much less $^{14}C$ is recovered in urine and feces suggesting that more BCIE or $^{14}C$ metabolites are exhaled (Smith, 1977). No additional metabolites were detected by these procedures. The simian species appears to form the glucuronide of 1-chloro-2-propanol more extensively than does the rodent.

Our current information on metabolic fate of these compounds is summarized as follows:

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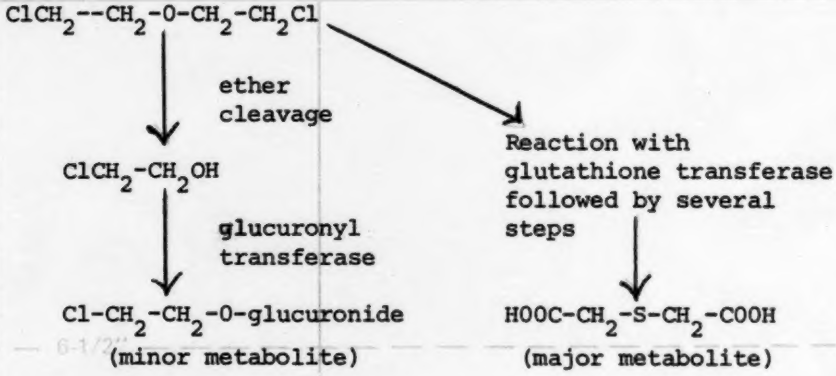
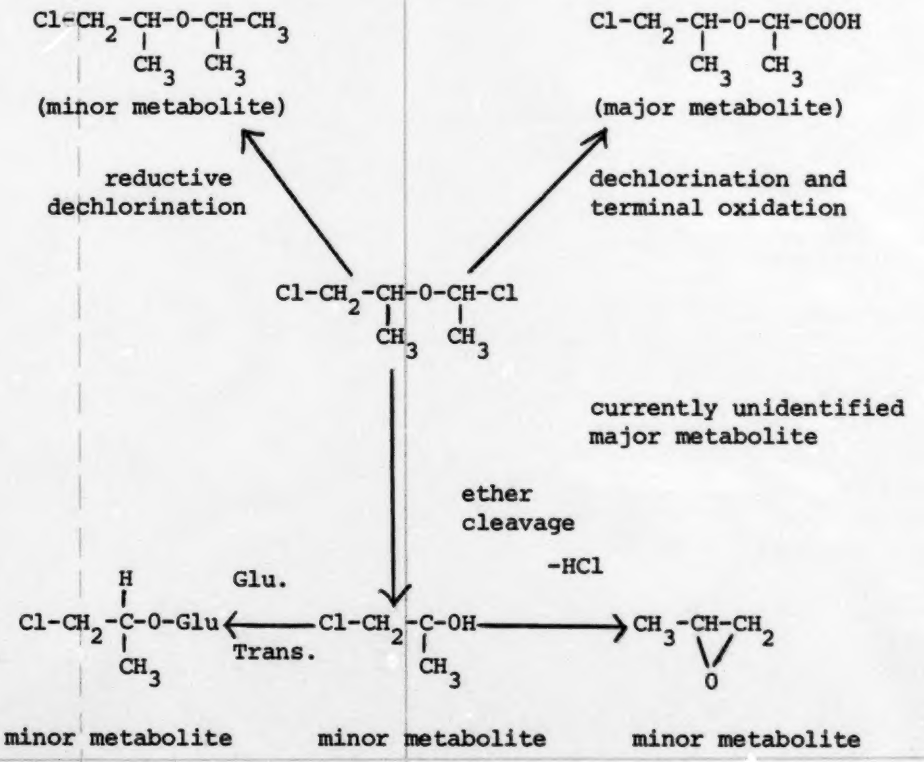
## BCEE


ClCH$_2$--CH$_2$-O-CH$_2$-CH$_2$Cl
ether cleavage
ClCH$_2$-CH$_2$OH
glucuronyl transferase
Cl-CH$_2$-CH$_2$-O-glucuronide
(minor metabolite)
Reaction with glutathione transferase followed by several steps
HOOC-CH$_2$-S-CH$_2$-COOH
(major metabolite)

## BCIE


Cl-CH$_2$-CH-O-CH-CH$_3$ (CH$_3$, CH$_3$)
(minor metabolite)
Cl-CH$_2$-CH-O-CH-COOH (CH$_3$, CH$_3$)
(major metabolite)
reductive dechlorination
dechlorination and terminal oxidation
Cl-CH$_2$-CH-O-CH-Cl (CH$_3$, CH$_3$)
currently unidentified major metabolite
ether cleavage
-HCl
H
Cl-CH$_2$-C-O-Glu
CH$_3$
Glu.
Trans.
Cl-CH$_2$-C-OH
CH$_3$
CH$_3$-CH-CH$_2$
O
minor metabolite
minor metabolite
minor metabolite

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